THE

American Journal of Physiology.

VOL. I. MARCH 1, 1898. NO. II.

THE INFLUENCE OF THE HEART-BEAT ON THE FLOW OF BLOOD THROUGH THE WALLS OF THE HEART.[1]

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IN 1689 J. Baptista Scaramucci[2] published two hypotheses which have played a great part in the history of the physiology of the heart: the first, that the deeper coronary vessels are squeezed empty by the contraction of the muscle fibres which surround them; and the second, that the coronary vessels are refilled from the aorta during the diastole of the heart. In 1707, Stroem[3] added to these the hypothesis that the coronary vessels are filled in diastole because their mouths are closed in systole by the semilunar valves. Thus, almost at the outset, the penetrating conception of Scaramucci was linked with an obstinate and overshadowing error, destined to be a source of controversy for nearly two hundred years.[4] Thebesius,

[1] The first account of my observations on the compression of the intramural vessels by the systole of the heart was published in the Journal of the Boston Society of Medical Sciences, No. 10, March 30, 1897. Most of the facts have been presented also to the American Physiological Society, in Washington, May 5, 1897, and to the British Association for the Advancement of Science, Toronto, 1897.

[2] SCARAMUCCI: Diario Parmense, 1689. Quoted by HALLER: Element. physiolog., 1778, lib. iv, s. v, p. 459.

[3] STROEM: Nova theoria machinæ animalis. Quoted by HALLER: *loc. cit.*

[4] A sufficient account of the literature of this subject has already been given by CERADINI, G.: Il meccanismo delle valvole semilunari del cuore. Milan, 1871: Der Mechanismus der halbmondförmigen Herzklappen. Leipzig, 1872. See also REBATEL, F.: Recherches expérimentales sur la circulation dans les artères coronaires. Paris, 1872.

Vieussens, Morgagni, Boerhaave, and other famous eighteenth century men took sides for and against the theory of Stroem, and not until the century had passed, and many observers of the first rank had shown that the mouths of the coronary arteries are often beyond the reach of the semilunar valves, and that the pulse in these vessels is synchronous with the pulse in the aorta, did opinion come to rest on the filling of the coronary arteries in both systole and diastole. The calm that followed was brief indeed. About 1840, Marshall Hall attempted to revive the old belief, but was answered by the experiments of Kleefeld. Five years after Kleefeld, the controversy broke out afresh. Brücke on the one side and Hyrtl on the other, neither knowing that he was repeating arguments and observations that already filled many pages of cardiac literature, fought over the old ground, drew many with them into an extended and often unprofitable discussion, — and reached the old conclusion. Once more physiological opinion settled to the belief that the coronary arteries are filled during systole as well as diastole, — a position since rendered impregnable by the observations of Ceradini and the experiments of Rebatel and of Martin and Sedgwick.

Throughout this long discussion, the primary hypothesis of Scaramucci, namely, that the deeper coronary vessels are emptied by the squeeze of the fibres contracting around them, received but scant attention. Thebesius,[1] in the celebrated inaugural dissertation in which he gave the first accurate description of the cardiac veins that bear his name, had said that in "no way could the arterial blood be forced into the vessels of the heart, unless during diastole; because in systole, the contraction of the fibres is so intense that all blood would be forced out, from the arteries no less than from the veins, — a condition that actually can be observed in the hearts of amphibia — frogs and others — which appear all white and bloodless when contracted, but are red and swollen with blood when relaxed in diastole;" and such reasoning was accepted by many who forgot that the heart of the frog is almost wholly wanting in bloodvessels, and that the red color of the full ventricle is due to the blood which fills the ventricle, seen through its translucent walls.

After the middle of the present century, experiment grew bolder and speculation began to yield place to direct observation. Hyrtl,[2] in 1855, trying to prove that the coronary arteries were filled in both

[1] Thebesius: De circulo sanguinis in corde. Leiden, 1708, p. 14.

[2] Hyrtl: Ueber die Selbststeuerung des Herzens. Vienna, 1855, p. 9.

systole and diastole, severed a coronary artery in the living rabbit, cat, and dog, and declared positively that only the upper segment spurted in systole, — a statement confirmed by Perls.[1] In 1876, Klug[2] drew a ligature about the rabbit's heart at the auriculo-ventricular junction while the heart was in full systole, and again while in diastole. He then coagulated the blood in the cardiac vessels by holding the organ some time in dilute sulphuric acid, and compared thin sections of the ventricle with regard to the amount of blood in their walls. The vessels of the heart ligated during diastole were filled with blood, while those of the heart ligated during systole contained little blood. But neither of these experiments can be said to be of value in our present inquiry: for the observation of Hyrtl, though accurate for his purpose, which was to determine which limb of the severed artery "spurted," is otherwise incorrect; and the method of Klug is open to objections based upon facts discovered since his time.

With regard to Hyrtl's work, it is true that the distal segment of a severed coronary artery does not "spurt," but it is also true, as will be shown in detail in the description of the writer's experiments, that blood is forced out of the distal segment with each contraction of the ventricle. The quantity which thus escapes is extremely small, but this is because the amount of blood contained in the distal segment of a severed "terminal" artery is always necessarily small. The anastomosis with neighboring vessels is too slight to permit of collateral circulation, and only a free collateral circulation can cause the distal end of a severed artery to bleed profusely.

Turning to Klug's experiment, let us consider first the heart ligated in systole. Klug slowed the heart in order to be sure of the moment of ligation. The reader will not need to be reminded that when the beat of the mammalian heart is considerably slowed by exhaustion, or by artificial means, as in Klug's method, the cavity of the ventricle is seldom, if ever, fully emptied. The observations of Pratt[3] have shown the ease with which the veins in the heart wall are filled from the cavity of the ventricle through the vessels of Thebesius. It is clear that even if a ligature could be drawn tight around the auriculo-ventricular junction in the precise fraction of a second during which the mammalian heart remains fully contracted, the relaxation of the

[1] Perls, M.: Archiv für pathologische Anatomie, 1867, xxxix, p. 189.

[2] Klug, F.: Centralblatt für die medicinische Wissenschaften, 1876, p. 134.

[3] Pratt, F. H.: The nutrition of the heart through the vessels of Thebesius and the coronary veins. American journal of physiology, 1898, i, p. 86.

ventricle after the tying of the ligature would fill its capillaries from the ventricular cavity, so that the amount of blood in the ventricular walls when the heart came to be examined would in no wise correspond to the amount present in the walls at the height of their contraction. If the heart is permitted to beat at its usual rapid rate, the ventricular cavities may be fully emptied at each stroke; but the time for the tying of the ligature is then so short that it is obviously impossible to be sure whether the ligation is made in full systole or a little before or after systole. If it were possible to be sure of the moment of ligation, and to make certain that the ventricular cavities were empty at that moment, and that the ligature shut off the auricles entirely, — the mural capillaries could still be filled when the heart relaxes from the blood in the large superficial coronary vessels, which are not within the grasp of the contracting fibres and cannot be compressed by them. In the heart ligated in diastole, it cannot be determined whether the blood found in the intramural vessels was present there at the moment of ligation, or entered the walls afterward through the veins of Thebesius or the superficial coronary vessels. Finally, the plunging of the fresh heart, warm from the body, into a coagulating bath of sulphuric acid, may so change the tonus of the ventricle as to alter materially the amount of blood in its capillaries. These sources of error render the observations of Klug unavailable.

It is Rebatel[1] whom we must thank for the first fruitful experiment in this field. Chauveau had given him the circulation in the coronary arteries as the subject of his inaugural dissertation, and had suggested that a T-tube should be placed in the right coronary artery of the horse and connected with an hæmodromograph, which should write a curve of the quickness of flow in the coronary artery, while at the same time a curve of the tension in the aorta should be recorded for purposes of comparison. Rebatel secured these curves, and saw at a glance that the beginnings of the upstrokes in the aortic and the coronary curves coincided exactly, showing that the blood-wave is synchronous in the two arteries, and that the coronary arteries are filled during systole. He saw also that the primary increase in the rapidity of current in the coronary artery was followed by a second augmentation "corresponding exactly to the moment when the aortic tension is least, *i. e.*, to the diastole of the heart. The first augmen-

[1] Rebatel, F.: Recherches expérimentales sur la circulation dans les artères coronaires. Paris, 1872.

tation," Rebatel asserts, "is evidently due to the propulsion imparted to the column of liquid by the contraction of the ventricle; the second current may be due to the entrance of a new wave from the aorta

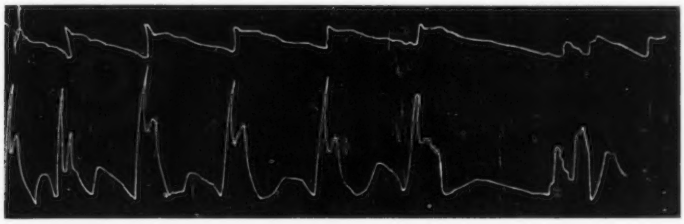

FIG. 1. Curves of the tension in the aorta (upper tracing) and the quickness of flow in the right coronary artery of the horse, simultaneously recorded (Rebatel's Fig. 3, page 25).

into the coronary artery, or to a sudden diminution of the peripheral resistance in the intramural vessels (p. 27)." To determine the origin of the second current, the tension and the quickness of flow in the coronary artery were recorded simultaneously. It was then seen that the tension curve was like that of every other artery, and presented no secondary rise or other feature that could account for the secondary augmentation in the quickness of flow. Thus led to a variation in the peripheral resistance, Rebatel concluded that the primary blood-wave

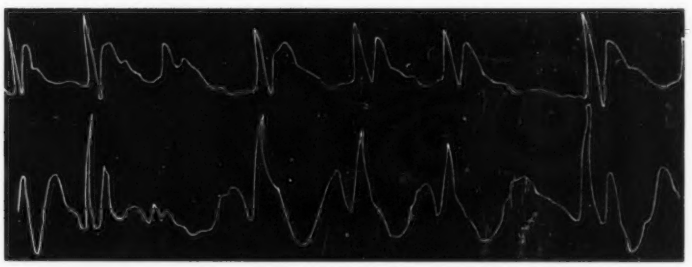

FIG. 2. Curves of the tension and quickness of flow in the right coronary artery of the horse, simultaneously recorded (Rebatel's Fig. 8, page 29).

penetrates with difficulty into the intramural branches during systole, because of their compression by the contracting cardiac muscle, but when the relaxation of the ventricle opens the peripheral vessels, the pent stream rushes suddenly forwards, and thus produces the second, or diastolic, rise in the curve of the hæmodromograph.

Rebatel himself does not accept this hypothesis unreservedly. His attitude is that of M. Marey,[1] who, on being shown the curves, admitted that the first proposition, namely, the filling of the coronary arteries in systole, is incontestable, while the second, namely, that the increase in quickness of flow is due to the opening of the intramural vessels by the relaxation of the ventricle is only "very probable and legitimately deduced." An analysis of Rebatel's tracings (Fig. 2) suggests that even this qualified approval was incautious. The extraordinary artificial irregularities in these curves at once attract attention. The curves are defaced by huge after-vibrations and inertia errors. The shock of the primary wave drives the writing levers far beyond the real maximum of the upstrokes; there is then a sharp rebound, which, in several instances, carries the writing points far below the correct level of the curve. Evidently the lever of the hæmodromograph, once set in motion by the sudden and violent changes in pressure and velocity consequent on the beat of the heart, has continued to swing. Serious as these faults are, they are by no means the chief reason for doubting the correctness of Rebatel's reasoning. According to him, the second augmentation is caused by the relaxation of the ventricle opening the compressed intramural vessels. But this relaxation occurs in the first half of the cardiac cycle, as shown by the position of the dicrotic notch in those of his tension curves that are written clearly enough to make the dicrotic notch visible. Hence, the maximum of the second augmentation, according to the hypothesis, should occur at the moment when the compressed vessels are opened by the swiftly relaxing ventricle, and not shortly before the ventricle contracts again, as in the curves before us. The delay cannot be explained by the sluggishness of the recording apparatus, for Rebatel assures us that the primary waves of tension and quickness are synchronous.

The form of the wave supposed to indicate a second augmentation of the rate of flow is still less reconcilable with Rebatel's hypothesis. The alleged increase in velocity quickly reaches its maximum, and is always succeeded by a rapid fall, greater in many of the tracings than the rise which precedes it. In the absence of any change in the blood-pressure either in the aorta or in the coronary arteries, it is impossible to understand how this slowing in the blood stream can take

[1] M. Marey, que nous remercions d'avoir bien voulu examiner nos tracés, admet, ainsi que nous, la première proposition comme incontestable, et la seconde comme très-probable et légitimement déduite (p. 31).

place. If a reservoir containing water kept at a constant level and provided with an elastic outflow tube is raised 130 cm. above the mouth of the tube, thus giving a constant pressure like that of the blood in the coronary arteries, shown to be constant by Rebatel's tension curves, and the mouth of the tube compressed, as the tubes of the coronary system are said by Rebatel to be compressed, and then released, while the water flowing out during two brief successive periods is measured, — it will be found that the outflow per unit of time, or in other words the velocity, is even a little greater in the second period than in the first. His second augmentation should, therefore, not have been followed by a marked slowing in the rate of flow.

Thus, seeing that Rebatel's second augmentation of velocity is not synchronous in his own curves with the relaxation said to be its cause, and perceiving that the form of the curve offered by him in evidence is physically improbable under the conditions premised by him, we may conclude that his results do not prove his assumption that the intramural vessels of the heart are compressed in systole.

I have spoken thus fully of Rebatel's work both because of its intrinsic interest and because his are the only recorded experiments that bear directly upon the problem in hand. It is true that Martin and Sedgwick,[1] ten years after Rebatel, recorded simultaneously curves of the blood-pressure in the carotid artery and in a branch of the left coronary artery; but their tracings were taken with a mercury manometer, and show nothing more than the synchronism in the primary pulse-wave, finer details being obscured by the inertia of the mercury.

I.

My own observations upon the characters of the coronary pulse began Sept. 16, 1895, with the record of the pressure-curve in the carotid and left coronary arteries in the dog. It seemed *a priori* probable that variations in the peripheral resistance in the coronary arteries would be visible in the pressure-curve, provided it were written with a sensitive manometer.

The heart of a dog anæsthetized with ether was exposed, the ramus descendens of the left coronary artery ligated about two centimetres from its origin, and a cannula tied into the central end. The

[1] MARTIN, H. N., and W. T. SEDGWICK: Journal of physiology, 1882, iii. p. 165.

cannula was then connected by thick-walled but flexible rubber tubing to a glass tube, which led to a sensitive Hürthle membrane manometer, placed on the level of the artery. Evidently a manometer thus situated must receive the pressure-changes in the ramus circumflexus of the left coronary artery and in the branches given off by the ramus descendens in the first part of its course, *i. e.*, between its origin and the cannula. A second manometer recorded simultaneously the changes of pressure in the carotid artery. But the hope of securing a curve from the coronary arteries differing from the pressure-curve

FIG. 3. Sept. 16, 1895. Curves of the blood-pressure in the left coronary artery (upper tracing) and the carotid artery (lower tracing) of the dog, recorded simultaneously. One half the original size. The horizontal line below each curve is the line of atmospheric pressure. In the case of the carotid artery, the atmospheric pressure line served also for the record of the time, in fifths of a second. The intervals of the graduation-scales correspond to a pressure of 20 mm. Hg. On raising the pressure in the Hürthle manometers to 100 mm. Hg. as here recorded, and then opening the chamber of the manometer to the pressure of the atmosphere, the writing points returned accurately to the line of atmospheric pressure, — this line in the pressure-scale being thus twice drawn. The vertical lines are synchronous ordinates. During the latter part of the curves, the heart was slowed by vagus excitation.

of other arteries was not realized. The most careful scrutiny of the two curves taken during the ordinary contractions and during the slowing of the heart by vagus excitation (see Fig. 3) failed to reveal any noteworthy difference, except that the pulse-wave reaches the coronary artery sooner than the carotid, depending of course on the nearness of the former vessel to the heart.

The first fully satisfactory evidence of the effect of the contraction of the ventricle on the flow of blood through the walls of the heart was secured during the writer's experiments on extirpated portions of the ventricle of the dog and cat. When a piece of the mammalian ventricle is kept beating by supplying it with defibrinated blood

through its nutrient artery at a constant pressure, each beat can be seen to force the blood out of the severed vessels in the margins of the fragment. The details of several of these experiments are as follows: —

Experiment March 29, 1897. A dog weighing 11 kilos, anæsthetized with morphia and ether, was bled from the left carotid artery, and the blood whipped, filtered through glass wool, and diluted with an equal volume of 0.8 per cent normal saline solution. Normal saline of the same strength was meanwhile allowed to flow into the right jugular vein. After a short interval, the dog was again bled from the carotid artery. A second injection of saline solution was followed by a third bleeding. The product of these bleedings was mixed, and placed in a reservoir at the temperature of the body. The heart was now extirpated, and a cannula tied into the ramus descendens of the left coronary artery not far from the apex of the left ventricle. That part of the apex which could be fed through the cannula was then excised. Both apex and basal portion fibrillated. The septum was removed. The piece of ventricle secured was 28 mm. in length (*i. e.* the direction from the base to the apex), 23 mm. broad opposite the end of the cannula, and 27 mm. broad at the somewhat flattened tip of the apex. The ventricle measured from base to apex 70 mm. The cannula was now connected with the blood reservoir and the apex perfused with blood. In a few moments regular and strong contractions set in. Curves were recorded with an ordinary muscle lever. The flow of blood from the veins was increased during each systole. The experiment was stopped after the apex had contracted one hour and forty minutes. During a part of this time the preparation was in a bath of blood at the temperature of the body.

March 30, 1897. On the morning of this day, a cannula was placed in the ramus descendens of a dog prepared as in the foregoing experiment, and most of the left ventricle and all of the right ventricle and septum except a fringe near the arteria descendens cut away. The portion remaining was fed through the cannula with defibrinated dog's blood, and beat strongly and at first quite regularly. It was observed that the outflow from the veins was increased at each systole. Distinct pulsations synchronous with the contractions of the heart-fragment were observed in the vena descendens at the point where it crosses the auriculo-ventricular groove. A cannula was tied into this vein, and a pulsation of the liquid in the cannula noted.

April 5, 1897. A pulse synchronous with systole was observed in the liquid in a cannula placed in the coronary artery of a piece of dog's ventricle fed with defibrinated blood from a reservoir at a constant pressure.

April 9, 1897. The circumflex area of the left ventricle of a cat's heart was fed with defibrinated cat's blood at a constant pressure through a cannula

placed in the ramus circumflexus. A vein on the surface of the ventricle was incised, and a little stream of normal saline solution allowed to flow over the opening, so as to prevent the blood collecting there. By this means a clear view of the wound in the vein and the escaping blood was secured. The discharge from the vein was then seen to be distinctly greater with each contraction of the ventricle. The superficial veins in a fragment of the auricle left attached to the preparation were observed to be nearly obliterated by each systole of the auricle. The pulse in these auricular veins could not have been caused by the rhythmic contractions of the coronary sinus, for the pulse in the veins continued after their separation from the sinus. Moreover, a similar pulse, noted in the superficial ventricular veins, ceased when the ventricle stopped beating, although the coronary sinus continued to contract.

The effect of the contraction of the heart on the contents of the intramural vessels can also be demonstrated in the living animal, as the next experiment will show.

April 12, 1897. A dog weighing 24 kilos was anæsthetized with morphia and ether, and the heart exposed by the resection of a part of the first five ribs on the left side. A branch of the vena descendens was incised about midway between the base and the apex of the ventricle, and a small stream of warm 0.8 per cent normal saline solution allowed to flow over the spot in order that the wound and the quantity of blood escaping from it might be readily seen. The vagus was now divided in the neck, and the peripheral end stimulated with induction shocks of such a strength that the ventricle was not continuously inhibited, but still gave occasional beats. Each time the ventricle contracted, the blood gushed from the vein. The increased outflow appeared absolutely synchronous with the contraction.

An eye-witness of this experiment could hardly have been persuaded that the gush of blood from the vein in systole was due to the transmission of the arterial pulse wave through the capillaries into the veins, yet it seemed advisable to answer this possible objection by direct experiment.

July 22, 1897. The experiment of April 12 was this day repeated, and again each contraction of the ventricle caused a greatly increased outflow from the vein. The vagus inhibition being prolonged, the heart swelled greatly, and the occasional contractions which broke through the inhibition were very strong. Each of these powerful contractions caused the blood to spurt from the vein. The heart was now excised, and the aorta connected with a reservoir of defibrinated dog's blood much diluted with 0.8 per cent

NaCl solution. The pressure in the reservoir was about 100 mm. Hg, so that, as soon as the connection with the aorta was made, the blood from the reservoir filled the artery, closed the semilunar valves, and passed through the coronary vessels. The perfused heart beat for a few minutes with considerable strength. With each beat the wound in the vein spurted, as an artery spurts when severed in the living animal.

The following experiments show that the squeezing of the vessels by the contracting muscle fibres makes itself evident in the arteries as well as in the veins. In this connection, it should be remarked once more, that the coronary arteries are "terminal arteries." In the absence of a collateral circulation, any pulsation observed in the distal segment of a coronary artery after its ligation is probably due to the compression of the intramural vessels by the contraction of the heart, and not to the transmission of an arterial pulse through collateral branches from other arteries.

November 18, 1897. A dog was anæsthetized with morphia and ether, and the heart exposed by the resection of the first five ribs on the left side. The ramus descendens was then ligated about 20 mm. from its origin. The artery was now incised 10–12 mm. distal to the ligature. A little blood escaped from the wound. On stimulating the vagus so that the ventricle contracted only occasionally, and allowing a small stream of warm normal saline solution to flow over the opening, it was possible to see plainly that each beat forced blood out of the artery. There was no visible delay between the beat and the outflow. The artery was now tied a few millimetres distal to the wound. The slight flow from the artery then ceased altogether, but during each systole a little blood appeared at the mouth of the wound in the artery.

The next day, a very high constant pressure was suddenly made in the aorta of a living dog, so that the semilunar valves were kept closed for a time, the pressure on their aortic side being greater than the maximum pressure in the left ventricle. The coronary circulation was fed during this time not by the beat of the ventricle but by the blood in the pressure-reservoir. Nevertheless, each beat of the ventricle forced blood out of the incision made in a coronary vein on the surface of the ventricle and out of a wound made in the arteria descendens distal to a ligature which had been placed around it. The details are as follows: —

November 19, 1897. The great vessels and heart of a dog anæsthetized with morphia and ether were exposed by resecting five ribs on the left and

three ribs on the right side and removing the upper part of the sternum. Cannulas were placed in the right and left carotid arteries. The right subclavian artery was ligated at its origin, and the left subclavian artery and the aorta prepared so that they could be clamped at the proper moment. The cannula in the left carotid artery was connected with a reservoir containing 0.8 per cent NaCl solution at a pressure of 140 mm. Hg. The cannula in the right carotid was connected to a mercury manometer, which showed a maximum pressure of 51 mm. Hg (the heart being rather feeble from long exposure). A vein on the surface of the left ventricle was now incised. The venous blood escaped from the wound in weak jets synchronous with the contractions of the ventricle, which were infrequent enough to permit the outflow to be seen distinctly. The stopcock between the carotid artery and the reservoir of saline solution under pressure was now opened and the left subclavian artery and the aorta clamped. The pressure in the manometer connected with the right carotid artery then rose to more than double its former maximum height. The semilunar valves were kept shut by this very high pressure in the aorta. The left ventricle, unable to open the semilunar valves, became greatly distended. An observation on the outflow from the incised artery and vein was made the moment the high pressure in the aorta closed the semilunar valves, before there could possibly have been time for the heart-beat to change sufficiently to overcome a pressure nearly three times as great as the former maximum arterial pressure, if indeed it could ever have done so. The blood still emerged from the vein in gentle systolic jets. The wound in the artery merely oozed, but the quantity escaping was distinctly greater in systole.

The emptying of the intramural vessels by the systolic squeeze of the fibres around them has been repeatedly observed in this Laboratory in the course of experiments on the extirpated heart of the cat, and has recently been admirably demonstrated by my friend, Mr. F. H. Pratt, by suspending a strip of the cat's heart, fed through one of the coronary arteries, in a large vessel of normal saline solution. The experiment is so instructive that it seems worth while to describe in this place a simple method by the aid of which the phenomena may be very easily shown.

A cat is anæsthetized with ether and cannulas placed in the left carotid artery and the right jugular vein. The animal is now bled from the artery. When the blood no longer flows except in drops, the artery is clamped, and 0.8 per cent NaCl solution at a temperature of 37° C. allowed to flow slowly into the jugular vein. When the blood vessels are well filled with saline solution, the cat is bled

again. The blood drawn in the first bleeding is diluted one half with the normal saline solution. The defibrinated blood mixture from both bleedings is then placed in a Mariotte tube, 30 cm. long and 3 cm. in diameter, of 190 c.c. capacity. The Mariotte tube opens below into a vertical glass tube about 5 mm. in diameter, on the end of which is a cannula provided with a stopcock. The cannula is inserted in the ramus descendens or the ramus circumflexus of the left coronary artery and all the heart cut away except that part of the ventricle supplied by the chosen artery. The fragment of the ventricle is now suspended in a very large beaker, filled with warm normal saline solution. When the Mariotte tube, the connecting tube, and the cannula are filled with the defibrinated blood, the height of the liquid column is about 65 cm., giving a blood-pressure of about 50 mm. Hg in the coronary artery. On opening the stopcock between the cannula and the upright tube, the blood circulates through the coronary artery and its branches, and the fragment of ventricle presently begins to beat. With each contraction the blood shoots from the severed vessels in the margins of the fragment some distance into the surrounding liquid, making a funnel-shaped cloud in the clear saline solution.

II.

Having thus demonstrated the pressure which the muscular fibres in the heart exercise upon the intramural vessels during systole, it remains to consider to what extent this constriction and subsequent relaxation assist the flow of blood through the heart walls. That they do assist the flow of blood through the heart walls seems *a priori* probable; it is, indeed, difficult to imagine how the periodical squeezing of vessels communicating on the one hand with the aorta, a reservoir in which the pressure is always relatively very high, and on the other with outflow channels in which the pressure is always relatively very low, could fail to drive the blood towards the point of low pressure, *i. e.* into the veins. But these premises do not justify the conclusion that the systolic compression of the intramural vessels increases the total volume of the coronary circulation. This is quite another problem, and one which cannot be answered from the data thus far brought forward. It has just been demonstrated that the circulation through the intramural vessels is diminished during the contraction of the fibres around them. The emptying of the vessels

and their subsequent refilling is favored by this same rhythmic contraction. Which of these factors has the upper hand? Does the check which the circulation through the walls sustains during systole diminish the total volume of blood passing through the wall per minute, or is the lessening more than made up by the favorable factors — the emptying of the intramural vessels and their easier refilling? The experiments next to be described afford a partial answer to this question.

In February, 1896, while studying with Messrs. Magrath and Kennedy the relation of the volume of the coronary circulation to the frequency and force of the ventricular contraction in the isolated heart of the cat, I observed that the heart took more blood through the coronary arteries from a reservoir under constant pressure when contracting than when at rest. The same observation was made again, later in that year, when at work with Miss Hyde on the effect of the distention of the ventricle on the flow of blood through the walls of the heart. The fact is very well demonstrated by Fig. 4.

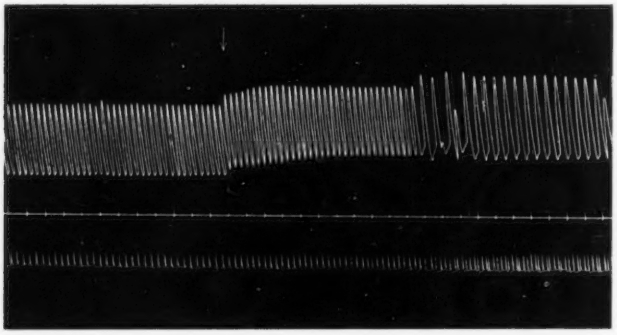

FIG. 4. Showing the increase in the volume of the coronary circulation consequent on an increase in the force of ventricular contraction. The uppermost tracing is the pressure in the left ventricle of the isolated heart of the cat, recorded by a Hürthle manometer; the next is the time, in seconds; and the lowermost is the record of the drops of blood flowing through the coronary vessels. The arrow points to the distention of the ventricle, which shortly calls forth beats of greater force. From an experiment performed with Miss I. H. Hyde.

In this experiment the extirpated heart of a cat was fed with warm defibrinated cat's blood from a reservoir at constant pressure through a cannula in the ascending aorta, all the branches of that vessel except the coronary arteries having previously been tied. The blood

passed from the coronary artery into the right ventricle, and thence through a glass tube, drop by drop, onto an aluminium plate fastened upon the lever of a Marey tambour.[1] The variation in the air pressure in the tambour occasioned by the falling drops was transmitted through a connecting tube to a second tambour, provided with a small chamber, thin membrane, and very light moving parts, and recorded by its writing lever upon the smoked paper of a kymograph. With this record of the number of drops of blood passing through the coronary vessels was written the pressure in the left ventricle, the cavity of which was filled with normal saline solution and connected with a sensitive Hürthle membrane manometer. A side branch led from this cannula to a Mariotte flask placed higher than the heart and filled with normal saline solution. When the stopcock leading to this flask was opened, the pressure in the left ventricle rose, as shown by the rise in the base line of the curve. After a few seconds the stimulus of the increased intracardiac pressure caused the ventricle to beat with greater force and the volume of the coronary circulation became greater, — and this in spite of a diminished frequency of contraction. Later, the pressure in the ventricle was lowered to that of the atmosphere, the ventricle contracted less vigorously, and the volume of the coronary circulation was correspondingly reduced.

A diminution in the volume of the coronary circulation in consequence of lessening the frequency of contraction is demonstrated by Fig. 5. The uppermost curve in this figure records the pressure in the left ventricle of the isolated heart of the cat, fed through the aorta and coronary vessels with defibrinated cat's blood at a constant pressure and temperature. The ventricle was filled with saline solution and connected with a Hürthle membrane manometer. The second curve was written by the armature of an electro-magnet placed in the primary circuit of a du Bois-Reymond inductorium. The heavy white line records the stimulation of the peripheral end of the vagus nerve with a weak induced current; the individual strokes of the armature are blended, owing to the slow speed of the smoked paper. The third curve marks the number of drops of blood flowing through the coronary vessels, the recording apparatus being that used for the experiment illustrated by Fig. 4. The fourth curve marks the time in seconds. The weak excitation of the vagus diminished the

[1] For the details of this method and a discussion of its sources of error, see MAGRATH and KENNEDY: Journal of experimental medicine, 1897, ii, p. 13.

frequency of ventricular contraction, but left the force unchanged. The volume of the coronary circulation lessened when the frequency of contraction lessened, and was restored with the restoration of the former frequency.

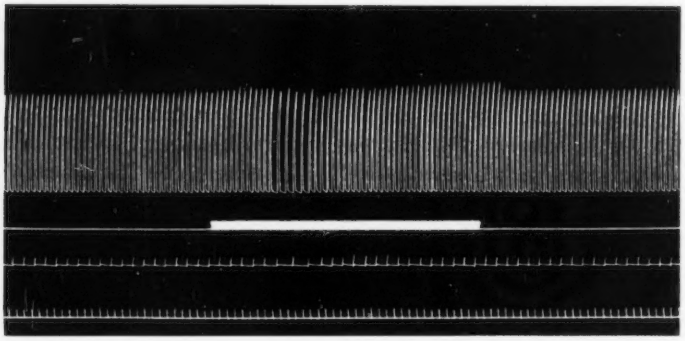

FIG. 5. March 26, 1896. Showing the lessening in the volume of the coronary circulation consequent on a lessening of the frequency of the heart-beat. The uppermost tracing is the pressure in the left ventricle of the isolated heart of the cat; the line below was drawn by the writing point of an electro-magnet placed in the primary circuit of the inductorium, the broad, white band indicating the duration of vagus stimulation; the next curve records the number of drops of blood passing through the coronary vessels; and the lowermost tracing is the time, in seconds. The weak excitation of the vagus finally lessens the frequency of contraction, — at once the volume of the coronary circulation is also lessened.

It would seem, then, that an increase in either the force or the frequency of the contractions of the heart increases the volume of blood passing through the coronary circulation by means of the periodical emptying of the intramural vessels, — yet it would not be prudent to accept this conclusion unreservedly. Two possible sources of error suggest themselves. The recorded changes in the volume of the coronary circulation may depend upon alterations in peripheral resistance in consequence of changes in the tonus of the heart muscle, or they may be due to changes in the vascular tonus in consequence of the action of vasomotor nerves. The first of these sources of error may be excluded with considerable certainty. The base line of the intraventricular pressure curves in Figs. 4 and 5 gives no evidence of changes in tonus; Fig. 5 is particularly convincing. The possible action of vasomotor nerves cannot be wholly excluded. Yet the pronounced synchronism between the changes

in frequency and the changes in the volume of the coronary circulation in Fig. 5 points toward a mechanical explanation, and seems to warrant the statement that the increase in the volume of the coronary circulation which accompanies an increase in the force or frequency of the heart-beat is probably to be explained by the periodical emptying of the intramural vessels by the contraction of the heart.

III.

It is conceivable that the emptying of the intramural vessels by the contraction of the heart may favor the flow of blood through the heart walls in two ways: first, by the diminished resistance which the empty patulous vessels should offer to the inflow of blood from the aorta when the heart relaxes; and second, by the suction which might accompany the sudden expansion of the compressed vessels, — expanding either by virtue of their intrinsic elasticity, or because of the pull of the surrounding tissues upon their walls, as the heart quickly regains its diastolic form. It will be best to begin with the second problem, namely, the possible suction of the relaxing heart muscle.

The method by which this problem was attacked consists in suddenly connecting the distal portion of a coronary artery of the strongly beating heart with a small reservoir of blood at the atmospheric pressure. If each compression of the deeper branches of the artery were followed by an expansion sufficient to cause a noteworthy suction, the blood in the reservoir should be drawn into the artery; for this blood is the sole source of supply throughout the experiment, the "terminal" nature of the coronary arteries preventing any material backflow from collateral branches. It will be seen from the experiments about to be cited that no appreciable suction can be demonstrated in the larger coronary arteries, even when a very sensitive minimum valve is interposed between the artery and the reservoir in order to prevent the possible masking of the suction by rising pressures accompanying the contraction of the ventricle.

April 14, 1897. The heart and great vessels of a dog anæsthetized with morphia and ether were exposed by the removal of a part of the chest wall. A glass cannula, 177 mm. long and 3.5 mm. in diameter, bent near the end as illustrated by Fig. 6, and furnished with a stopcock and a side branch leading to a minimum manometer, as shown in Fig. 7, was connected with a

reservoir containing warm defibrinated dog's blood, obtained in the manner described in the Exp. March 29, page 153. The pressure in the blood reservoir was maintained at a constant level of about 80 mm. Hg. (The exact reading of the mercury manometer connected with the reservoir was inadvertently omitted from the protocol.) The minimum valve and its manometer were filled with 0.8 per cent NaCl solution, and the cannula and the connecting tubes with defibrinated dog's blood. The long cannula was now rapidly passed through the innominate artery, aorta, and left coronary artery into the ramus circumflexus, which it filled completely, and the stopcock leading to the blood reservoir opened. The stopcock leading to the minimum manometer had previously been closed. The defibrinated blood entered the artery at about the normal temperature and pressure and maintained a satisfactory circulation. The heart continued to beat strongly and regularly. The blood reservoir was now suddenly shut off, and the stopcock leading to the minimum manometer as suddenly opened. The contents of the manometer passed slowly into the artery, but on comparing the level of the liquid in the manometer, with that of the heart it was found that the manometer was higher than the heart. The slow emptying of the manometer may therefore have been due to gravity. The experiment shows at least that there is no strong suction, otherwise the manometer would have been emptied rapidly.

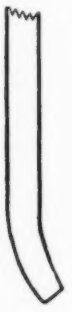

FIG. 6. Lower end of glass cannula for perfusing the ramus circumflexus in the living animal.

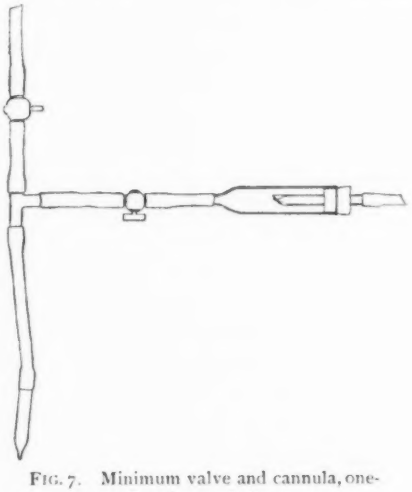

FIG. 7. Minimum valve and cannula, one-fourth actual size.

April 15, 1897. The foregoing experiment was repeated, but no suction could be demonstrated.

April 16, 1897. The experiment was varied by tying a cannula into the ramus descendens, opened for the purpose on the surface of the ventricle near the origin of the artery, and connecting this cannula with a minimum manometer, filled, as before, with normal saline solution. But no suction could be found.

April 23, 1897. A cannula tied into the ramus descendens of a dog's heart was furnished with a T-tube, one limb of which led through a minimum manometer to a nearly horizontal glass tube filled with normal saline solution, while the other led to a reservoir from which the descendens area was supplied with warmed, defibrinated dog's blood at about the normal pressure (Fig. 7). While the heart was beating well, the descendens area being fed from the pressure flask, the latter was suddenly cut off and the stopcock leading to the minimum valve tube opened. There was no suction, although the conditions of the experiment were all favorable to its discovery.

Experiments similar to that of April 16 on the dog have been tried on four cat's hearts (Nov. 11–17), but also without finding any suction.

It should be remarked that these are all negative results. Against a single positive result they would be worthless. Yet I am obliged at present to conclude that the relaxation of the heart wall does not produce a suction in the larger coronary vessels.

Having failed to demonstrate any suction in the coronary arteries during the diastole of the heart, it is necessary to accept the alternative explanation of the favorable influence of the heart-beat on the flow of blood through the heart-walls, namely, the diminished resistance which the empty patulous vessels offer to the inflow of blood when the heart relaxes.

Summary.

1. Curves of the blood-pressure in the carotid and the coronary artery, recorded simultaneously by two sensitive membrane manometers, reveal no noteworthy difference in the form of the pulse-wave.

2. The intramural branches of the coronary vessels are compressed by the contraction of the muscle fibres around them.

3. The volume of blood passing through the coronary vessels is increased by an increase in either the force or the frequency of the heart-beat.

4. It is probable that this increase in the volume of blood passing through the coronary vessels is accomplished largely through the periodical emptying of the intramural vessels by the systolic squeeze of the fibres around them.

5. The emptying of the intramural vessels by the contraction of the heart favors the flow of blood through the heart-walls chiefly by the diminished resistance which the empty patulous vessels offer to the inflow from the aorta when the heart relaxes.

6. The relaxation of the heart-walls does not produce a noteworthy suction in the larger coronary vessels.

A FURTHER STUDY OF THE INFLUENCE OF ALCOHOL AND ALCOHOLIC DRINKS UPON DIGESTION, WITH SPECIAL REFERENCE TO SECRETION.[1]

By R. H. CHITTENDEN, LAFAYETTE B. MENDEL, and HOLMES C. JACKSON.

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IN a previous paper[2] on the "Influence of Alcohol and Alcoholic Drinks upon the Chemical Processes of Digestion" it was carefully pointed out that any complete and satisfactory answer to the question "How do alcoholic fluids affect digestion?" cannot be obtained by any single line of experimentation, since the rate and extent of digestion may be modified in a variety of ways and through a variety of channels. Thus, due consideration must be given not only to the direct influence of alcoholic fluids upon the solvent or digestive power of the several digestive juices, but heed must also be given to the quantitative and qualitative modifications which the secretions themselves may undergo, as well as to variations in the rate of absorption and to the possible interaction of these and other factors. In our earlier paper, the data presented threw light only upon the character and extent of the influence exerted by various alcoholic fluids upon the purely chemical processes of digestion, *i. e.*, upon amylolysis and proteolysis. In the continuation of these studies during the past year our efforts have been directed mainly to acquiring a fuller knowledge of the action of alcoholic beverages upon secretion; and in so doing new data have been obtained which, it is hoped, will prove of value in explaining more fully the action of these fluids upon the whole process of digestion.

SALIVARY SECRETION.

The current statements regarding the influence of alcohol on the secretion of saliva are confined to a brief reference to the direct

[1] Being a statement of some research work done for the Committee of Fifty for the Investigation of the Liquor Problem, and to be regarded as a preliminary report, contributing facts upon which a general discussion may in the future be undertaken by the Committee as a whole.

[2] CHITTENDEN and MENDEL: American journal of the medical sciences. 1896, January–April.

action on the flow into the mouth. Thus it is stated that almost coincident with the burning sensation caused by alcohol taken into the mouth, a copious flow of saliva begins, due to reflex stimulation of the glands through the nervous system.[1] We have performed experiments with the object of ascertaining (1) the possible variations in the amount of salivary flow due to the presence of alcoholic fluids in the mouth, psychical influences being eliminated so far as possible; (2) the character of the saliva thus secreted; (3) the influence upon secretion of alcoholic beverages introduced into the stomach. It seemed particularly desirable to investigate this latter phase in view of the asserted influence of irritating substances (vinegar, alcoholic extract of pepper, etc.) when introduced directly into the alimentary tract through a fistula. There is said to result under such conditions a reflex flow of saliva, the nervous impulses being transmitted through the vagus.[2]

The Influence of Alcoholic Fluids introduced into the Mouth.—In the following experiments the attempt was made to ascertain something as to the character and extent of the direct stimulation of the salivary glands provoked by the presence of alcoholic fluids in the mouth, as well as to determine what quantitative changes, if any, may be called forth in the composition of the secretion in this way. These experiments were made on both man and dogs. The method, in the first instance, consisted in taking into the mouth 10 c.c. of the fluid studied, and allowing it to remain there for an instant previous to swallowing it. The normal conditions were thus closely imitated, and reflex influences from the stomach not excluded. The head was now turned to one side and rested upon the arm, the saliva being allowed to collect in the cavity of the mouth. As the fluid accumulated it was from time to time, during fifteen to twenty minutes, allowed to flow out of a corner of the mouth into a measuring vessel. Movements of the jaws and tongue were carefully avoided and psychic stimulation was excluded as far as possible. The method, already recommended by Hofbauer,[3] was found to be reasonably satisfactory, and control trials showed that the quantities of saliva obtained within periods of fifteen or twenty minutes could be appropriately compared.

[1] Compare, for example, KÜHNE: Lehrbuch der physiol. Chemie, 1868, p. 2; LAUDER BRUNTON: Disorders of digestion, 1886, p. 143.

[2] OEHL: Comptes rendus, lix, p. 336, quoted by HEIDENHAIN, Hermann's Handbuch der Physiologie, 1883, v, p. 83.

[3] HOFBAUER: Archiv fur die ges. Physiol., 1897, lxv, p. 503.

Of the saliva thus collected, 3–4 c.c. were taken for analysis. A weighed quantity was dried in a tared crucible on a water-bath and then for four or five hours at 105° C., this time being found sufficient to bring crucible and contents to a constant weight. Total solids were thus determined. The crucible was then ignited, care being taken to prevent loss by volatilization of salts. The ash thus obtained is given as salts in the protocols, while the organic constituents were obtained by subtracting the amount of salts from the total solids. In some cases the amount of Cl in the ash was determined by the usual method of titration with weak silver nitrate solution. The analytical results are all expressed in percentages. The following figures serve to illustrate the results of a typical duplicate analysis: —

SUBMAXILLARY SALIVA OF DOG.

	Water.	Total solids.	Organic constituents.	Salts.	Chlorine.
A.	98.99	1.01	0.80	0.21	0.042
B.	98.99	1.01	0.78	0.23	0.040

It is an observation easily verified, that the presence of a small quantity of strong alcohol or alcoholic beverage in the mouth excites a sudden flow of saliva. This acceleration in flow is, at most, a very brief one, and the rate of flow quickly returns to that pertaining to normal conditions, *i. e.*, absence of stimuli in the mouth. The stimulation in this case is not due merely to the mechanical action of the fluid introduced, nor is it a form of stimulation specific for alcohol alone, as our experiments on dogs have demonstrated. Thus, animals were anæsthetized with ether and chloroform through a tracheal cannula (thereby avoiding direct stimulation of salivary flow), a small dose of morphine, or a larger one of chloral, having been previously administered. A cannula was then introduced into one or both ducts of the submaxillary glands. A small wad of absorbent cotton moistened with the fluid to be studied was introduced with a forceps into the back of the mouth upon the tongue, and the flow of saliva from the ends of the cannulas noted. It was found by this method that water or weak sodium chloride solution (0.7 per cent) produced no further effect than the secretion of a drop or two of

SALIVARY EXPERIMENTS ON MAN.

	I.		II.		III.		IV.		V.		VI.		VI.		VIII.	
	water *a*	water *b*	water *a*	water *b*	water *a*	brandy *b*	water *a*	brandy *b*	water *a*	brandy *b*	water *a*	brandy *b*	water *a*	gin *b*	water *a*	sherry *b*
Amount collected in c.c. per 10 minutes.	4.0	4.0	4.4	3.7	2.7	5.3	3.8	4.4	4.7	8.0	4.4	7.1	4.0	4.6	3.5	4.4
Water, per cent.	99.49	99.57	99.52	99.54	99.51	99.49	99.50	99.40	99.57	99.19	99.56	99.45	99.57	99.51	99.41	99.39
Total solids, per cent.	0.51	0.43	0.48	0.46	0.49	0.51	0.50	0.60	0.43	0.81	0.44	0.55	0.43	0.49	0.59	0.61
Organic constituents, per cent.	0.36	0.31	0.35	0.33	0.33	0.35	0.35	0.45	0.31	0.58	0.30	0.38	0.31	0.35	0.41	0.43
Salts, per cent.	0.15	0.12	0.13	0.13	0.16	0.16	0.15	0.15	0.12	0.23	0.14	0.17	0.12	0.14	0.18	0.18
Salts calculated on total solids, per cent.	29.0	29.0	28.0	28.0	32.0	32.0	30.0	25.0	29.0	28.0	31.0	31.0	28.0	28.0	30.0	29.0

saliva due to the mere mechanical action of introducing the wad; with increasing strengths of salt the secretion was decidedly accelerated, flowing readily after application of 20 per cent salt solution, the acceleration, however, being very brief in duration (5 min.). The buccal cavity could be swabbed out with water occasionally, the effect being a minimal one. It was found that *weak* alcohol, introduced in this way, provoked little, if any, flow; while stronger alcohol (50 per cent) gave rise to a transitory secretion, the stimulation in this case, however, being far more marked than can be produced by the indirect action of alcohol through the stomach. Thus, in one animal, in which the activity of the glands was found pronounced when a drop of dilute acetic acid was applied to the tongue, injection of 100 c.c. 50 per cent alcohol directly into the stomach, failed to provoke any reflex salivary flow in half an hour.

Turning now to the influence of alcoholic fluids upon the rate of flow and composition of the saliva in man, the accompanying experiments, by the method above indicated, may be cited (p. 167). The first two (I. and II.) show the results obtained with successive portions of water; in the following ones, a control experiment with water in each instance preceded the trial with the alcoholic fluid.

The alcoholic content of the fluids employed was as follows: Brandy, 47 per cent by vol.; gin, 51 per cent; sherry, 21 per cent.

From these figures it is seen that the results obtained with two successive portions of water scarcely differ from each other, the tendency however being towards decreased flow accompanied by decrease in dissolved material in the saliva. Interpreted in physiological terms, these results indicate that the second stimulation with water is, if anything, weaker than its predecessor. In decided contrast appear the results obtained with the alcoholic liquors. Here may be observed an increased flow of saliva, not pronounced, but accompanied by an increase in both organic and inorganic constituents. The effect is precisely analogous, both in composition and rate of flow, to that brought about by an increase in intensity of stimulation, when the salivary glands are electrically excited through their nerves.[1]

The following diagram represents in graphic form the results given in the preceding table, *i. e.* (1) the relative rate of flow induced by water and by the alcoholic fluid; (2) the content of solid matter,

[1] Cf. HEIDENHAIN: Archiv für die ges. Physiol., 1878, xvii, p. 7, and Hermann's Handbuch der Physiologie, v, p. 52.

together with the relative proportion of ash or inorganic matter and of organic matter as indicated by the loss on ignition.

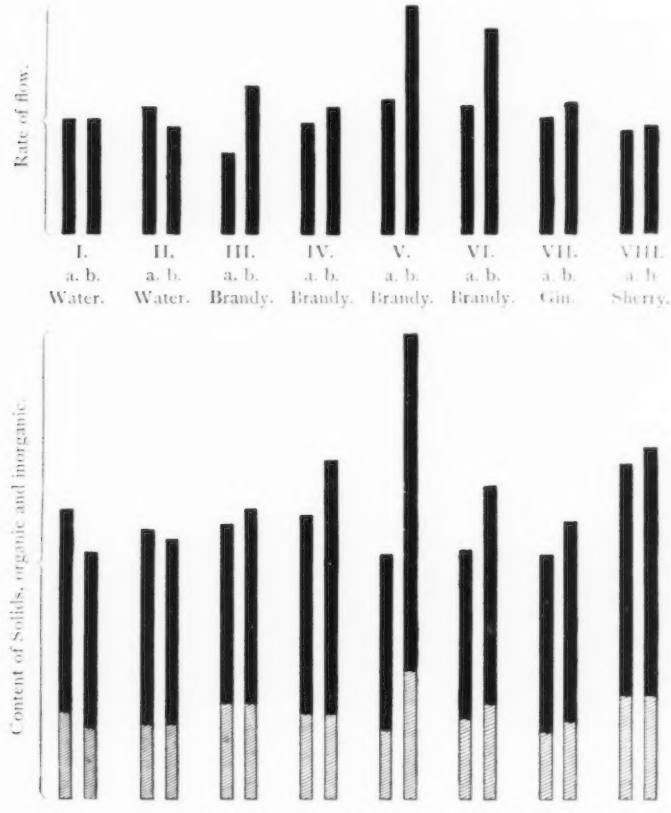

Diagram illustrating the relative influence of alcoholic fluids on the rate of secretion and composition of human saliva.

The Influence of Alcoholic Liquors introduced directly into the Stomach. — In our experiments on the reflex stimulation of salivary flow, the attempt to produce a persisting secretion due to the presence of alcohol in the stomach was unsuccessful; nor have we been able to obtain evidence of an unusual flow of saliva under such circumstances in dogs with gastric fistulæ. It seemed desirable, however, to examine the possible direct influence of alcoholic fluids on the salivary glands

and the resulting secretion, when other factors were excluded as far as possible. In these experiments dogs of 10 to 18 kilos were used. Chloroform-ether mixture was employed to produce anæsthesia, and was administered through a tracheal tube in part of the experiments, the danger of respiratory difficulties resulting from salivary flow induced in the glands as a result of the ether stimulation being thus avoided. In the later stages of the experiments the alcohol introduced usually sufficed to maintain the animal in perfect quiet. Fredericq [1] has recommended the use of alcohol for producing narcosis in rabbits; it has been found quite satisfactory for this purpose in the dog, the effects passing off with relative rapidity.

A glass cannula, bent at the end, was tied in Wharton's duct (and occasionally a second cannula into the duct of the sublingual gland). The chordo-lingual nerve was ligatured and cut at some distance centrally to the point where the chorda tympani branches off to the glands. All secretion in the corresponding gland was thus stopped except during stimulation of the chorda, which was accomplished through raising the peripheral end of the cut nerve by the ligature and slipping hook electrodes under it. The interrupted current of a du Bois induction-coil with a single element was used as the stimulus. Saliva was collected in small graduated cylinders. Alcohol was introduced into the stomach by making an incision through the linea alba, etc., and injecting the fluid directly into the organ thus exposed by means of a large needle-pointed syringe. By careful avoidance of the larger gastric vessels, bleeding was minimal. The general course of the experiments was as follows: A distance between the primary and secondary coil of the inductorium was selected such as a preliminary trial showed to give a medium rate of flow. This stimulus was, so far as possible, kept constant throughout the experiment. The chorda was repeatedly stimulated for periods of one minute, followed by pauses of two minutes, during which the nerve was kept covered. In this way sufficient quantities of saliva for analysis were collected. Before collecting a sample of saliva under any given conditions, the six or seven drops first discharged were thrown away, and thus the fluid stored up from previous stimulation in the gland lumina, ducts, and cannula was avoided.[2] After collecting two or three control samples, the fluid to be considered (usually warmed slightly) was injected

[1] FREDERICQ: Manipulations de physiologie, p. 19.

[2] Cf. HEIDENHAIN: Hermann's Handbuch der Physiologie, v, p. 53; LANGLEY and FLETCHER: Philosophical transactions, 1889, clxxx, B., p. 112.

into the stomach in the manner already described, and this was followed by a pause of five minutes. The pulse was observed at frequent intervals to detect any possible influence on the heart's action and consequent blood-flow. The samples of saliva collected were analyzed in the manner already described. At the conclusion of the experiment, the animal was killed by bleeding, and the condition of the glands, as well as of the stomach and other organs, examined. The protocols of three typical experiments are given below.

1, iii, 1897. Dog. Weight 14 kilos. Chloroform and ether administered through tracheal tube during part of the experiment. Distance of secondary coil = 200 mm. Period of stimulation = 1 min., followed by a pause of 2 min.

	Time.	Amount saliva collected c.c.	Rate of secretion per min. c.c.	Water. per cent.	Total solids. per cent.	Organic matter. per cent.	Salts. per cent.	Chlorine. per cent
I[1]	10.35	5.3	0.7	98.76	1.24	0.94	0.30	0.026
II	11.08	5.4	0.6	98.94	1.06	0.73	0.33	0.036
III	11.38	5.2	0.8	98.95	1.05	0.69	0.36	0.044
IV	11.56	4.0	0.8	98.90	1.10	....	....	0.048
	12.20	80 c.c. 50 per cent alcohol injected into stomach.						
V	12.35	4.8	0.8	98.96	1.04	0.69	0.35	0.047
VI	12.55	4.8	0.8	99.01	0.99	0.59	0.40	0.076
	1.15	100 c.c. 50 per cent alcohol injected into stomach.						
VII	1.21	4.9	0.8	99.05	0.95	0.59	0.36	0.055
VIII	1.42	6.0	1.0	99.05	0.95	0.60	0.35	0.060
IX	2.02	5.5	0.9	99.14	0.86	0.52	0.34	0.048
X	2.24	5.2	0.8	99.17	0.83	0.47	0.36	0.042
	2.53	100 c.c. 50 per cent alcohol injected into stomach.						
XI	2.58	4.5	0.6	99.07	0.93	0.63	0.30	0.034
XII	3.27	6.0	0.6	99.18	0.82	0.53	0.29	0.037
XIII	4.10	5.0	0.7	99.17	0.83	0.49	0.34	0.038

Dog killed. Stomach mucosa normal in appearance. Urinary bladder and gall bladder greatly distended. Stomach contents = 450 c.c., faintly acid in reaction, and containing 24.6 grams of alcohol. No food present.

[1] In this first period the distance of the secondary coil was 280 mm., but the stimulation was unsatisfactory.

22, iii, 1897. Bitch. Weight 10 kilos. Chloroform and ether administered during operation. Tracheotomy performed after operation. Distance of secondary coil = 240 mm. Period of stimulation = 1 min., followed by 2 min. pause.

	Time.	Amount saliva collected c.c.	Rate of secretion per min. c.c.	Water. per cent.	Total solids. per cent.	Organic matter. per cent.	Salts. per cent.	Chlorine. per cent.
I	11.30	4.6	1.1	98.68	1.32	1.03	0.29	0.032
II	11.42	4.7	0.9	98.70	1.30	0.96	0.34	0.074
III	11.57	4.0	0.7	98.84	1.16	0.73	0.43	0.146
	12.35	150 c.c. burgundy injected into stomach.						
IV	12.41	4.9	0.8	98.72	1.28	0.91	0.37	0.092
V	12.59	5.5	0.6	98.78	1.22	0.87	0.35	0.096
VI	1.29	4.7	0.7	98.91	1.09	0.82	0.27	0.071
	2.00	200 c.c. burgundy injected into stomach.						
VII	2.06	4.7	0.6	98.88	1.12	0.82	0.30	0.058
VIII	2.32	....	....	98.98	1.02	0.69	0.33	0.099

Dog killed; stomach contents = 190 c.c.; claret color; mucosa not inflamed. Contents contained 13.1 grams of alcohol. The burgundy used contained 5.2 per cent of alcohol.

Experiments of the character indicated by these protocols were carried out with alcohol in varying doses, whiskey, brandy, and wine, and control experiments with water were also made. In attempting to interpret the analytical data thus obtained in experiments extending over several hours it is necessary to bear in mind facts regarding salivary secretion which seem to be sufficiently established. Ludwig[1] showed that the submaxillary saliva secreted during stimulation of the chorda tympani undergoes a change in composition varying with the duration of the flow, the content of organic solids decreasing in far greater degree than the dissolved salts. Heidenhain[2] found that the percentage of salts in the saliva varies directly with the rate of secretion, quite independently of the state of the gland, the organic constituents, however, being influenced by the condition of the secreting organ as well as by the strength of stimulus and

[1] Ludwig and Becher: Zeitschr. f. rat. Med., 1851, N. F. i. p. 278. Cf. also Heidenhain: Hermann's Handbuch der Physiologie, v, pp. 47–49.

[2] Heidenhain: Archiv für die ges. Physiol., 1878, xvii, pp. 4 and 6.

12, iv, 1897. Bitch. Weight 9 kilos. Chloroform and ether during operation. Distance of secondary coil = 190 mm. Stimulation 1 min., followed by a pause of 2 min.

	Time.	Amount saliva collected c.c.	Rate of secretion per min. c.c.	Water. per cent.	Total solids. per cent.	Organic matter. per cent.	Salts. per cent.	Chlorine per cent.
I	9.24	4.5	0.9	98.76	1.24	0.97	0.27	0.062
II	9.40	4.6	0.7	98.89	1.11	0.81	0.30	0.054
	10.40	100 c.c. distilled water injected into stomach.						
III	10.53	4.7	0.6	99.04	0.96	0.66	0.30	0.049
IV	11.21	5.0	0.5	99.09	0.91	0.60	0.31	0.060
	11.50	100 c.c. distilled water injected into stomach.						
V	11.56	4.5	0.5	99.30	0.70	0.54	0.16	0.024
VI	12.25	4.5	0.6	99.33	0.67	0.36	0.31	0.078
VII	12.51	4.6	0.7	99.39	0.61	0.36	0.25	0.063
	1.18	100 c.c. 50 per cent alcohol injected into stomach.						
VIII	1.23	5.7	0.7	99.35	0.65	0.36	0.29	0.067
IX	1.44	4.8[a]	0.8	99.38	0.62	0.32	0.30	0.087
X	2.03	4.7	0.7	99.47	0.53	0.29	0.24	0.087
XI	2.25	4.7	0.6	99.47	0.53	0.22	0.31	0.097

Dog killed. Stomach mucosa normal. Contents = 100 c.c. No odor of alcohol.

resulting rate of secretion. These observations, verified by Werther[1] and by Langley and Fletcher,[2] have been extended by the latter investigators, who formulated the opinion that "the secretion of organic substances depends wholly, or almost wholly, upon the strength of the stimulus, whilst the secretion of water and of salts depends also upon the amount of blood flowing through the gland."[3] In view of the well-known fact that changes in the strength of the stimulus immediately bring about a change in both rate of secretion and composition of the saliva, we have attempted to maintain a constant stimulus throughout each series of observations by selecting some satisfactory distance of the secondary coil of the inductorium and by applying the electrodes as uniformly as possible. Owing to

[1] Werther: Archiv f. d. ges. Physiol., 1886, xxxviii, p. 293.

[2] Langley and Fletcher: *loc. cit.*, p. 152. [3] *Ibid.*, p. 132.

the gradual decline in the irritability of the exposed nerve, the impossibility of applying the electrodes constantly in one position, and other unavoidable difficulties, ideal results cannot be obtained. However, the difficulties were present in every experiment and the results are therefore more or less comparable.

An examination of the data obtained in the manner above indicated shows no constant appreciable influence of alcohol or alcoholic fluids upon the *rate of secretion* of submaxillary (or sublingual) saliva under the influence of a constant external stimulus. Even large doses of alcohol, sufficient to produce prolonged narcosis, fail to check the salivary flow, a result in striking contrast to the effects which morphine may bring about when used in moderately large doses. We have not infrequently observed, in other experiments, an entire absence of salivary flow even with very strong stimuli, when morphine was unintentionally given in doses larger than were necessary to produce a mild narcosis. On the other hand, there is likewise an absence of any stimulating action on the glands, in our experiments; at least the slight variations in the rate of flow after alcohol is administered are no greater than those brought about by water alone (cf. third protocol above). On the *total solids* likewise, the presence of alcohol seems to exercise no noticeable influence. There is a tendency toward decrease in amount as the experiments progress; this decrease, however, is entirely confined to the *organic constituents* of the saliva, the *salts* remaining comparatively constant in amount, as can be seen in the protocols above. The decrease in organic substances is in no way to be attributed to alcohol, since it may be obtained with water alone (cf. protocol third), or in the course of any protracted salivary secretion. Nor is this decrease remarkable when it is remembered that a small gland weighing a few grams has furnished 50 to 75 grams of saliva in the course of three or four hours. The organic constituents of the cells must thus be exhausted somewhat more rapidly than the anabolic processes of the gland can replace them, while the salts are obtained with relative ease from the blood. Any effect upon the secretion of inorganic salts such as might result in accordance with Langley's law (cf. p. 173) was not observed. A large number of determinations of the alkalinity of the saliva (towards lacmoid) likewise failed to show any constant relations. It is interesting in this connection to note that the submaxillary saliva of the

dog was always found alkaline to phenolphthalein, litmus, lacmoid, and methylorange. Mixed human saliva, like the bile of a number of animals, is almost always acid toward phenolphthalein.[1]

Gastric Secretion.

It has already been pointed out that in an accurate and complete study of the influence of alcohol and alcoholic drinks upon gastric digestion, no single line of experimentation can lead to full and concise results covering the whole ground of inquiry. It was therefore deemed advisable, for experimental purposes, to study the subject under several distinct heads, as (1) the influence of alcohol and alcoholic drinks upon the process of secretion; (2) upon the processes of absorption; (3) upon the motor functions of the alimentary canal; and (4) upon the purely chemical processes of gastric digestion. The last phase has already been considered at some length.[2]

The older announcements regarding the influence of alcohol are summarized in the statement that it is a strong stimulant of gastric secretion, and alcohol is recommended as a means of obtaining gastric juice from fistulæ in animals.[3] Larger doses are regarded as detrimental to the stomach, giving rise to transudation of alkaline fluid, — a process evidently pathological.[4] Gluzinski[5] found in experiments on man with brandy and dilute alcohol that these liquors gave rise, after a brief preliminary period, to the formation of a very active secretion rich in hydrochloric acid.

Likewise Wolff[6] states that cognac in small doses increases the secretion of hydrochloric acid, while in larger quantity it decreases the acidity of the gastric juice and retards peptone formation. The stomach fails to respond in a positive way, however, after the continued use of alcohol. While Klemperer[7] failed to note more than

[1] Chittenden: The reactions of some animal fluids. Science, N. S., v, p. 902.

[2] Chittenden and Mendel: *loc. cit.*

[3] Cf. Frerichs: Wagner's Handwörterbuch der Physiologie, 1846, iii, (1), p. 788; Kühne: Lehrbuch, pp. 28, 30; Heidenhain: Hermann's Handbuch der Physiologie, v, p. 115.

[4] Cf. Heidenhain: *loc. cit.*; Lauder Brunton: Disorders of digestion, 1886, p. 144.

[5] Gluzinski: Deutsches Archiv f. klin. Med., 1886, xxxix, p. 405. See Jahresbericht für Thierchemie, 1886, xvi, p. 263.

[6] Wolff: Zeitschr. f. klin. Med., 1889, xvi, p. 222; Jahresbericht f. Thierchemie, 1889, xix, p. 266.

[7] Klemperer: Zeitschr. f. klin. Med., 1890, xvii, Supp., p. 324: Centralbl. f. med. Wissen., 1891, p. 751.

a very slight increase in secretion resulting from moderate doses of alcohol, Blumenau[1] observed that 25–50 per cent alcohol introduced into the healthy human stomach acts as a secretory stimulant, bringing about an increased flow of gastric juice with rise of acidity after a period of 2–3 hours. More recently Brandl[2] has found in experiments on fistulous dogs that alcohol — as contrasted with water introduced with food stuffs into the stomach — brings about an unfailing, though not particularly large, increase in gastric secretion. With repeated and increasing doses of alcohol, Haan[3] has further observed an augmentation of acidity in the dog, followed by a diminution in the amount of secretion and a gradual decline in acidity after several doses.

In our first series of experiments on gastric secretion, attention was directed to the volume and acidity resulting from the introduction of alcoholic fluids into the stomach, independently of any stimulating action due to food simultaneously introduced. Dogs in fasting condition were employed in every instance, and morphine sulphate (introduced subcutaneously) followed by chloroform-ether was used preparatory to operative interference. The method consisted in ligating the duodenum just beyond the pylorus and then introducing a definite volume of the fluid to be examined into the empty stomach in the manner already indicated in previous experiments. In several cases, dogs with gastric fistulæ were employed. The abdomen was quickly sewed up after this operation, chloroform-ether stopped, and the animal allowed entire freedom of movement. The liquid employed was ordinarily warmed gently to avoid the asserted stimulating action of cold fluids on the gastric mucosa.[4] Ligations of the œsophagus and œsophageal fistulæ were avoided, since a somewhat extended experience with gastric fistula dogs, as well as the experiments about to be described, have convinced us, in agreement with Heidenhain's observations,[5] that under ordinary circumstances, *i. e.* in the absence of unusual stimuli (and with slightly narcotized animals) the amount of saliva secreted is small at most, and fails to induce any pronounced secretion in the stomach.[6] Further, we have

[1] BLUMENAU: Therapeutische Monatshefte, 1890, v, p. 504; Jahresbericht f. Thierchemie, 1891, xxi, p. 212.

[2] BRANDL: Zeitschr. f. Biologie, 1892, xxix, p. 304.

[3] HAAN: Comptes rendus de la société de biologie, 1895, ii, p. 817.

[4] Cf. KÜHNE: Lehrbuch der physiol. Chemie, p. 28.

[5] Hermann's Handbuch, v, p. 112.

[6] Compare also the experiment described on page 168.

found that an unusual flow of saliva is at once readily detected by the physical character of the stomach contents, *e. g.* frothing, etc. Furthermore, the conditions of our experiments were intended to approach those normally obtaining in the body as nearly as possible; and finally, a sufficient number of control experiments in which water was introduced into the stomach, have left no doubt as to the validity of the method. At the end of three to four hours — a period shown by our experiments to cover the digestion time of a test meal for the dog — the animal was bled to death, the œsophagus ligated at the lower end, the stomach removed from the body, wiped free from blood, and the contents discharged into a graduated vessel. In the fluid thus obtained total acidity, free and combined HCl, and acid reacting salts were determined by the method of Töpfer;[1] alcohol was estimated, when present, in the distillate from a definite portion of the gastric contents, by the pycnometer method; total solids were determined by drying a weighed quantity of fluid in a tared crucible at 100–105 C°. Protocols follow: —

A. **Control Experiments with Water:** —

I. 31 v, 1897. Dog, with gastric fistula, well healed. Weight 21 kilos. Fluid removed completely through fistula.

Introduced 200 c.c. *distilled water* at 10 50 A. M.
Contents removed at 1.55 P. M. = $3\frac{1}{2}$ hrs.

Volume of fluid recovered from stomach = 160 c.c. = **80 per cent** of original volume.
Analysis of the contents gave:

Total acidity	0.203 per cent.[2]
Free HCl	0.192
Loosely combined HCl	0.002
Salts	0.009
Total solids	0.624

II. 28 vi, 1897. Dog, with gastric fistula, well healed. Weight 25 kilos. Fluid removed completely through fistula.

Introduced 135 c.c. *distilled water* at 11 A. M.
Contents removed at 1.45 P. M. = $2\frac{1}{4}$ hrs.

Volume of fluid recovered from stomach = 110 c.c. = **81 per cent** of original volume.
Analysis of the contents gave:

Total acidity	0.274 per cent.
Free HCl	0.241
Loosely combined HCl	0.018
Salts	0.015
Total solids	0.77

[1] Töpfer: Zeitschr. f. physiol. Chemie, 1894, xix. p. 104.
[2] Expressed as HCl in all the experiments.

III. 24 v, 1897. Dog. Weight 7.7 kilos.

Introduced 125 c.c. *distilled water* at 10 A. M.
Contents removed at 1.50 P. M. = $3\frac{5}{6}$ hours.
Volume of fluid recovered from stomach = 114 c.c. = **91 per cent** of original volume.

Analysis of the contents gave:

Total acidity	0.094 per cent.
Free HCl	0.065
Loosely combined HCl	0.004
Salts	0.025
Total solids	0.47

IV. 29 v, 1897. Dog. Weight 14.5 kilos.

Introduced 200 c.c. *distilled water* at 9.30 A. M.
Contents removed at 1.15 P. M. = $3\frac{3}{4}$ hrs.
Volume of fluid recovered from stomach = 206 c.c. = **103 per cent** of original volume.[1]

Analysis of the contents gave:

Total acidity	0.047 per cent.
Free HCl	0.040
Loosely combined HCl	0.004
Salts	0.003
Total solids	0.50

V. 2 vi, 1897. Dog. Weight 10.5 kilos.

Introduced 125 c.c. *carbonated water* at 9 A. M.
Contents removed at 12.45 P. M. = $3\frac{3}{4}$ hrs.
Volume of fluid recovered from stomach = 125 c.c. = **100 per cent** of original volume.

Analysis of the contents gave:

Total acidity	0.191 per cent.
Free HCl	0.152
Loosely combined HCl	0.014
Salts	0.025
Total solids	0.55

In this experiment the CO_2 was completely absorbed.

VI. 1 vii, 1897. Dog. Weight 10 kilos.

Introduced 76 c.c. of 2 per cent *dextrose* solution at 9.10 A. M.
Contents removed at 12.40 P. M. = $3\frac{1}{2}$ hrs.
Volume of fluid recovered from stomach = 68 c. c. = **90 per cent** of original volume.

Analysis of the contents gave:

Total acidity	0.072 per cent.
Free HCl	0.047
Loosely combined HCl	0.007
Salts	0.018

[1] A small quantity of saliva doubtless found its way into the stomach, as the dog salivated somewhat at the beginning of the operation and the stomach contents had a frothy appearance.

B. **Experiments with strong Ethyl Alcohol**: —

VII. 17 v, 1897. Dog. Weight 23 kilos.

Introduced 200 c.c. of 37 per cent *alcohol* at 10.45 A. M.
Contents removed at 2.15 P. M. = $3\frac{1}{2}$ hrs.
Volume of fluid recovered from stomach = 407 c.c. = **203 per cent** of original volume.

Analysis of the contents gave:

Total acidity	0.164 per cent.
Free HCl	0.112
Loosely combined HCl	0.043
Salts	0.009

VIII. 31 v, 1897. Dog. Weight 21 kilos. Gastric fistula well healed.

Contrast experiment with water and alcohol.

α. The first part of this experiment has been described under I. p. 177.

β. After discharge of previous stomach contents completely through fistula, 200 c.c. $37\frac{1}{4}$ per cent *alcohol* were introduced into the stomach through fistula at 1.55 P. M.
Contents removed at 5 P. M. = $3\frac{1}{12}$ hrs.
Volume of fluid recovered from stomach = 460 c.c. = **230 per cent** of original volume.[1]

Analysis of the contents gave:

Total acidity	0.220 per cent.
Free HCl	0.164
Loosely combined HCl	0.011
Salts	0.045
Total solids	0.987

C. **Experiments with weak (5 per cent) Ethyl Alcohol**: —

IX. 24 vi, 1897. Bitch. Weight 8 kilos.

Introduced 100 c.c. 5 per cent *alcohol* at 10.45 A. M.
Contents removed at 2 P. M. = $3\frac{1}{4}$ hrs.
Volume of fluid recovered from stomach = 110 c.c. = **110 per cent** of original volume.

Analysis of the stomach contents gave:

Total acidity	0.119 per cent.
Free HCl	0.086
Loosely combined HCl	0.011
Salts	0.022
Total solids	0.69

X. 8 vi, 1897. Bitch. Weight 7.3 kilos.

Introduced 110 c.c. 4.8 per cent *alcohol* at 9 A. M.
Contents removed at 12.45 P. M. = $3\frac{1}{4}$ hrs.
Volume of fluid recovered from stomach = 135 c.c. = **123 per cent** of original volume.

Analysis of the stomach contents gave:

Total acidity	0.202 per cent.
Free HCl	0.148
Loosely combined HCl	0.021
Salts	0.033

[1] A post-mortem examination showed that the stomach contents could be completely discharged through the fistula by the method adopted.

The results of the foregoing experiments, expressed in percentages, are combined in the following table.

A. With water.	Relative volume of fluid at end of experiment.	Total acidity.	Loosely combined HCl.	Free HCl.	Salts.	Total solids.
I	80	0.203	0.002	0.192	0.009	0.62
II	81	0.274	0.018	0.241	0.015	0.77
III	91	0.094	0.004	0.065	0.025	0.47
IV	103	0.047	0.004	0.040	0.003	0.50
V	100	0.191	0.014	0.152	0.025	0.55
VI	90	0.072	0.007	0.047	0.018	....
Average.	90.8	0.147	0.008	0.123	0.016	0.58

B. With strong alcohol.	Relative volume of fluid at end of experiment.	Total acidity.	Loosely combined HCl.	Free HCl.	Salts.	Total solids.
VII	203	0.164	0.043	0.112	0.009	....
VIII	230	0.220	0.011	0.164	0.045	0.99
Average.	216.5	0.192	0.027	0.138	0.026	0.99

C. With weak alcohol.	Relative volume of fluid at end of experiment.	Total acidity.	Loosely combined HCl.	Free HCl.	Salts.	Total solids.
IX	110	0.119	0.011	0.086	0.022	0.69
X	123	0.202	0.021	0.148	0.033	....
Average.	116.5	0.160	0.016	0.117	0.027	0.69

A glance at the data presented leaves little doubt as to the pronounced stimulating action of pure ethyl alcohol upon gastric secre-

tion, even with solutions of only five per cent strength. The effect is not merely one characterized by the discharge of water into the stomach cavity, but gives evidence of a true secretory process. Thus, the volume of fluid found after introduction of water into the stomach is not increased, there being rather a tendency in the opposite direction. Edkins,[1] v. Mering,[2] and others have shown that the absorption of water from the stomach is practically *nil*, while the absorption of alcohol goes on quite rapidly. In our own experiments, the alcohol used had entirely disappeared from the stomach in the course of the experiments; the question of absorption will, however, be referred to in another connection. With five per cent alcohol the increase in the volume of the gastric contents is noticeable, becoming very pronounced with the stronger percentages of alcohol. The increase in total solids gives confirmation of stimulated secretion, as does also the increase in acidity. It must be remembered, further, that the increase in acidity shown by the figures is a relative one; expressed absolutely in grams, the total acid secreted is obviously increased in far greater degree than the percentage figures indicate. The specific action of alcohol is strikingly shown in Experiment VIII., in which the conditions permitted of comparative experiments with water and alcohol on the same animal, with the following results: —

COMPARISON OF THE TWO EXPERIMENTS (VIII. *a*, *β*).

Fluid introduced in stomach.	Fluid recovered from stomach after 3 hours.	Relative volume. per cent.	Total acidity.	Free HCl.	Loosely combined HCl.	Salts.	Total solids.
200 c.c. water	160 c.c.	80	0.203	0.192	0.002	0.009	0.624
200 c.c. alcohol (37½ per cent.)	460 c.c.	230	0.220	0.164	0.011	0.045	0.987

A comparison of the proteolytic activity of the two secretions by Grützner's carmine-fibrin method showed a decidedly greater digestive power in the case of the "water" secretion. Much stress cannot be placed, however, on a single experiment. The gastric fluids obtained in the experiments with alcohol possessed strong proteolytic properties in every case examined.

[1] Edkins: Journal of physiology, 1892, xiii, p. 445.

[2] v. Mering: Verhandlungen des XII. Congresses f. innere Medicin, Wiesbaden, 1893; Therapeutische Monatshefte, 1893, vii, p. 201.

In view of this pronounced action of alcohol on gastric secretion it seemed desirable to ascertain something more definite regarding the way in which this process is provoked. The control experiments with water gave evidence that the mere contact of the fluid with the stomach mucosa could not be the cause of gastric stimulation. It will be remembered that even vigorous mechanical stimulation or irritation ordinarily fails to yield more than a few grams of secretion,[1] — an observation in decided contrast to the phenomena of gastric flow during the presence of digestible materials in the stomach. The following experiments throw light on the question raised: —

XI. 25 v, 1897. Dog. Weight 23 kilos. The intestine was ligatured just beyond the pylorus. Another ligature was applied below the point of entrance of the duct of Wirsung. 20 c.c. of 60 per cent alcohol were injected into the lumen of the intestine between these ligatures, while 105 c.c. of 60 per cent alcohol were introduced into the intestine beyond the second ligature. Then

Introduced 200 c.c. *water* into stomach at 10.45 A. M.
Contents removed at 2.30 P. M. = $3\frac{3}{4}$ hrs.

Volume of fluid recovered from stomach = 260 c.c. = **130 per cent** of original volume.

Analysis of stomach contents gave:

Total acidity	0.241 per cent.
Free HCl	0.213
Loosely combined HCl	0.002
Salts	0.026

XII. 28 v, 1897. Bitch. Weight 28 kilos. Intestine ligatured just beyond the pylorus. Another ligature was applied below the point of entrance of the duct of Wirsung. 125 c.c. of 60 per cent alcohol were injected into the lumen of the intestine below the second ligature,[2] then

Introduced 200 c.c. *water* into stomach at 11 A. M.
Contents removed at 2.45 P.M. = $3\frac{3}{4}$ hours.

Volume of fluid recovered from stomach = 375 c.c. = **187.5 per cent** of original volume.

Analysis of stomach contents gave:

Total acidity	0.333 per cent.
Free HCl	0.306
Loosely combined HCl	0.004
Salts	0.023
Total solids	0.30

[1] Cf. TIEDEMANN and GMELIN: Die Verdauung nach Versuchen, 1831, p. 92; SCHIFF: Leçons sur la physiologie de la digestion, ii, p. 244.

[2] The return of alcoholic fluid into the stomach was thus absolutely prevented.

SUMMARY OF RESULTS OF EXPERIMENTS.

No.	Relative volume of fluid at end of experiment.	Total acidity.	Loosely combined HCl.	Free HCl.	Salts.	Total solids.
XI	130.0	0.241	0.002	0.213	0.026	....
XII	187.5	0.333	0.004	0.306	0.023	0.30
Average.	158.5	0.287	0.003	0.259	0.024	0.30

From these data it seems clear that a stimulation of the gastric glands may take place, independently of any *direct* gastric irritation, in consequence of the influence of alcohol absorbed from the intestine. The volume of the fluid in the stomach increased relatively far more than when five per cent alcohol was introduced directly into the stomach (cf. Experiments IX., X., p. 179). The composition of the fluid (high acidity, free HCl, total solids) likewise gives evidence of active secretion, while the fluid was found to be strongly proteolytic. The absorption of the alcohol was complete in these experiments; and when it is remembered how quickly alcohol is distributed and disappears in the body, the actual amount reaching the gastric glands must have been relatively small, or at least must have acted during a brief period only. It seems probable, therefore, that there occurs here an indirect stimulation quite comparable to that resulting after absorption of peptone from the alimentary tract, and it is interesting to note by way of comparison that Khigine,[1] in his experiments upon the isolated fundus of the dog, found that the acidity of the secretion after absorption of digestion products runs parallel to a certain degree with the increase in volume. Whether the absorbed alcohol acts directly upon elements of the gastric mucosa (Heidenhain's "secondary secretion"), or becomes a stimulus to specific secretory nerve fibres (Khigine), we are unable at present to decide.[2]

In connection with this "secondary" secretion of gastric juice due to the presence of alcohol in the small intestine, it is to be noted that Macfadyen, Nencki, and Sieber[3] found among the bacteria normally

[1] KHIGINE: Archives des sciences biologiques. St. Petersbourg, 1895, iii, p. 461.

[2] Cf. HOWELL: American text-book of physiology, 1896, p. 182.

[3] MACFADYEN, NENCKI, and SIEBER: Archiv f. experimentelle Pathologie und Pharmakologie, 1891, xxviii, p. 311.

present in this portion of the alimentary canal species which give rise to a production of ethyl alcohol from carbohydrates ingested.

D. **Experiments with Alcoholic Beverages.** — It might naturally be assumed that the action of the various alcoholic beverages on gastric secretion would be similar, qualitatively, to that of their common constituent ethyl alcohol. Previous investigation, however, has shown that the influence of these liquors on the purely chemical processes of digestion is not necessarily proportionate to their content of alcohol,[1] hence it seemed desirable to study the effect of a number of typical liquors on secretion, by the method of the previous experiments. This we have done with the following results: —

XIII. 21 vi, 1897. Dog. Weight 10.7 kilos.

Introduced 50 c.c. **sherry** + 25 c.c. *water* (14 per cent alcohol) at 10.20 A. M.
Contents removed at 2.15 P. M. = $3\frac{1}{12}$ hrs.

Volume of fluid recovered from stomach = 160 c.c. = **213 per cent** original volume.

Analysis of stomach contents gave:

Total acidity	0.367 per cent.
Free HCl	0.300
Loosely combined HCl	0.020
Salts	0.047
Total solids	1.72

XIV. 2 vi, 1897. Dog. Weight 18.5 kilos.

Introduced 50 c.c. **whiskey** + 100 c.c. *water* (16 per cent alcohol) at 11.15 A. M.
Contents removed at 3 P. M. = $3\frac{3}{4}$ hours.

Volume of fluid recovered from stomach = 320 c.c. = **213 per cent** original volume.

Analysis of stomach contents gave:

Total acidity	0.382 per cent.
Free HCl	0.346
Loosely combined HCl	0.011
Salts	0.025
Total solids	0.42

XV. 3 vi, 1897. Bitch. Weight 8 kilos.

Introduced 125 c.c. **Hochheimer** (13.3 per cent alcohol) at 10 A. M.
Contents removed at 1.45 P. M. = $3\frac{3}{4}$ hrs.

Volume of fluid recovered from stomach = 140 c.c. = **112 per cent** original volume.

Analysis of stomach contents gave:

Total acidity	0.230 per cent.
Free HCl	0.165
Loosely combined HCl	0.038
Salts	0.027

[1] CHITTENDEN and MENDEL: *loc. cit.*

XVI. 28 vi. 1897. Dog. Weight 25 kilos. Gastric fistula well healed.

Contrast experiment with water and wine.

α. The first part of this experiment has been described under II. p. 177.

β. After complete discharge of previous stomach contents through the fistula, 135 c.c. **white wine** were introduced into stomach through fistula at 1.45 P. M.

Contents removed at 4.30 P. M. = 2¼ hrs.

Volume of fluid recovered from stomach = 170 c.c. = **126 per cent** original volume.

Analysis of stomach contents gave:

Total acidity	0.425 per cent.
Free HCl	0.342
Loosely combined HCl	0.018
Salts	0.065
Total solids	1.79

XVII. 23 vi. 1897. Dog. Weight 12.3 kilos.

Introduced 125 c.c. **claret** (5.15 per cent alcohol) at 9.30 A. M.

Contents removed at 1.30 P. M. = 4 hrs.

Volume of fluid recovered from stomach = 225 c.c. = **180 per cent** original volume.

Analysis of stomach contents gave:

Total acidity	0.373 per cent.
Free HCl	0.324
Loosely combined HCl	0.025
Salts	0.024
Total solids	1.90

XVIII. 18 vi. 1897. Bitch. Weight 10.2 kilos.

Introduced 100 c.c. **lager beer** (4 to 5 per cent alcohol) at 10.20 A. M.

Contents removed at 2.15 P. M. = 3¼ hours.

Volume of fluid recovered from stomach = 110 c.c. = **110 per cent** original volume.

Analysis of stomach contents gave:

Total acidity	0.357 per cent.
Free HCl	0.241
Loosely combined HCl	0.064
Salts	0.052
Total solids	9.26

XIX. 23 vi. 1897. Dog. Weight 10 kilos.

Introduced 100 c.c. **lager beer** (4.5 per cent alcohol) at 10.10 A. M.

Contents removed at 2 P. M. = 3⅞ hrs.

Volume of fluid recovered from stomach = 125 c.c. = **125 per cent** original volume.

Analysis of stomach contents gave:

Total acidity	0.241 per cent.
Free HCl	0.169
Loosely combined HCl	0.032
Salts	0.040
Total solids	5.51

XX. 14 vi, 1897. Dog. Weight 14 kilos.

Introduced 150 c.c. **porter** (3.75 per cent. alcohol) at 9.45 A. M.
Contents removed at 1.30 P. M. = $3\frac{3}{4}$ hrs.

Volume of fluid recovered from stomach = 195 c.c. = **127 per cent** original volume.

Analysis of stomach contents gave:

Total acidity	0.371 per cent.
Free HCl	0.320
Loosely combined HCl	0.036
Salts	0.015
Total solids	2.19

XXI. 7 vi, 1897. Bitch. Weight 8.5 kilos.

Introduced 125 c.c. **lager beer** (4.7 per cent alcohol) at 10.15 A. M.
Contents removed at 2.10 P. M. = $3\frac{1}{2}$ hrs.

Volume of fluid recovered from stomach = 285 c.c. = **228 per cent** original volume.

Analysis of stomach contents gave:

Total acidity	0.378 per cent.
Free HCl	0.308
Loosely combined HCl	0.016
Salts	0.054
Total solids	2.88

XXII. 14 vi, 1897. Dog. Weight 8.2 kilos.

Introduced 150 c.c. **porter residue** [1] at 11.30 A. M.
Contents removed at 3.15 P. M. = $3\frac{1}{4}$ hrs.

Volume of fluid recovered from stomach = 135 c.c. = **90 per cent** original volume.

Analysis of stomach contents gave:

Total acidity	0.352 per cent.
Free HCl	0.280
Loosely combined HCl	0.014
Salts	0.058
Total solids	2.29

XXIII. 9 vi, 1897. Dog. Weight 10 kilos.

Introduced 130 c.c. **lager beer residue** [2] at 10.30 A. M.
Contents removed at 2.30 P. M. = 4 hrs.

Volume of fluid recovered from stomach = 175 c.c. = **134 per cent** original volume.

Analysis of stomach contents gave:

Total acidity	0.346 per cent.
Free HCl	0.270
Loosely combined HCl	0.038
Salts	0.038
Total solids	6.80

[1] The residue left on evaporation of 150 c.c. porter, dissolved in 150 c.c. distilled water.

[2] Residue from evaporation of 130 c.c. beer, dissolved in 130 c.c. water.

For the sake of comparison these data are contrasted in the following table: —

	Relative volume of fluid at end of experiment.	Total acidity.	Loosely combined HCl.	Free HCl.	Salts.	Total solids.
XIV. Whiskey + H_2O (16% alcohol)	213	0.382	0.011	0.346	0.025	0.42
XIII. Sherry + H_2O . (13% alcohol)	213	0.367	0.020	0.300	0.047	1.72
XV. White wine . . (13% alcohol)	112	0.230	0.038	0.165	0.027	[illegible]
XVI. White wine . . (13% alcohol)	126	0.425	0.018	0.342	0.065	1.79
XVII. Claret . . . . (10% alcohol)	180	0.373	0.025	0.324	0.024	1.80
XVIII. Beer . . . . . (4.7% alcohol)	110	0.357	0.064	0.241	0.052	9.26
XIX. Beer . . . . . (4% alcohol)	125	0.241	0.032	0.169	0.040	5.51
XXI. Beer . . . . . (4.7% alcohol)	228	0.378	0.016	0.308	0.054	2.88
XXIII. Residue of Beer (like XXI.)	134	0.346	0.038	0.270	0.038	6.80
XX. Porter . . . . . (5.3% alcohol)	127	0.371	0.036	0.320	0.015	2.19
XXII. Residue of porter (like XX.)	90	0.352	0.014	0.280	0.058	2.29

These results afford tangible evidence of the stimulating action of the liquors examined, as shown in the increased volume of gastric contents, accompanied by increase in acidity. That alcohol is an important factor in the production of these phenomena seems certain. Contrast, for example, Experiment XX. with XXII., which differs only in the absence of the alcohol. But the wines and malted beverages contain a variety of other constituents such as organic acids,[1] which perhaps contribute to increase the stimulating effect, and are doubtless partly responsible in a number of experiments for the high acidity observed. The contrast between the action of water and wine is strikingly shown in Experiments XVI. *a* and *β* carried out on the same animal.

[1] Cf. CHITTENDEN and MENDEL: *loc. cit.*, pp. 56, 80.

COMPARISON OF THE TWO EXPERIMENTS (XVI. *α*, *β*).

Fluid introduced in stomach.	Fluid removed from stomach after 3 hours.	Relative volume. per cent.	Total acidity.	Free HCl.	Loosely combined HCl.	Salts.	Total solids.
135 c.c. water	110 c.c.	81	0.274	0.241	0.018	0.015	0.77
135 c.c. white wine	170 c.c.	126	0.425	0.342	0.018	0.065	1.79

The marked increase in total solids in many of these experiments, however, is not to be attributed, as it is in the case of pure alcohol, entirely to the increased secretion; it is rather in part accounted for by the unabsorbed constituents of the liquor employed. The following table, compiled from analyses at hand, shows that a large portion of the total solids in the gastric juices obtained may be derived from other sources than the secretion itself: —

TABLE SHOWING TOTAL SOLIDS OF GASTRIC CONTENTS.

Nature of fluid introduced into stomach.	Total solids introduced into stomach.	Total solids in gastric contents at end of experiment.
II. Water	0 grams.	0.84 grams.
IX. Weak alcohol . .	0 "	0.69 "
VIII. Strong alcohol . .	0 "	4.50 "
XIV. Whiskey	0.15 "	1.34 "
XVI. White wine . . .	2.8 "	2.41 "
XVII. Claret	3.9 "	4.28 "
XIII. Sherry	2.35 "	2.78 "
XVIII. Beer	7.0 "	10.00 "
XXIII. Beer residue . . .	9.1 "	11.56 "
XX. Porter	6.6 "	4.16 "
XXII. Porter residue . .	6.6 "	3.10 "

E. **Character of the Gastric Juice obtained by Stimulation with Alcohol.** — The gastric juice obtained as a result of the stimulating influence of alcohol and alcoholic liquors resembles that ordinarily

procured from gastric fistulæ in its physical characters; it is a thin, colorless, or very faintly yellow fluid containing occasional flocks of mucus in suspension. There was no evidence of irritation or hyperæmia of the mucosa, and all traces of blood were absent. After the doses used, the gastric lining was of a pale or faintly pink color when removed after bleeding the animal. When colored alcoholic liquors were employed, the gastric contents retained the characteristic coloring matter, the latter not being absorbed, while the alcohol entirely disappeared. In chemical composition, the gastric juice appeared somewhat more acid than that ordinarily secreted. It likewise contained a larger amount of solid matter, and in harmony with this fact the proportion of combined hydrochloric acid was increased, which in turn suggests the presence of a somewhat larger amount of proteid or other like matter. The fluids were repeatedly tested with boiled fibrin for proteolytic action, and this was always found vigorous. In the experiments in which alcohol was introduced directly into the intestine (Experiments XI., XII., p. 182), the intestinal lining was not abnormal in appearance, the reaction being alkaline to litmus in the upper duodenum and neutral or faintly alkaline further along the alimentary canal. This corresponds with the observations on the normal reaction of the intestinal contents of the dog, by Moore and Rockwood,[1] whose statements we have repeatedly verified.

Gastric Digestion.

Since chemical, mechanical, and physiological processes go on side by side during digestion, we have carried out a series of experiments to determine in what way and to what extent the factors already investigated combine or coöperate under the influence of alcohol and alcoholic liquors. Our method has included the examination of the stomach contents after test meals were given. The statements current in the literature on this subject are by no means concordant.

In experiments on a woman having a gastric fistula Kretschy[2] observed that alcohol retarded digestion. Buchner[3] found that in

[1] Moore and Rockwood: Journal of physiology, 1897, xxi, p. 373.

[2] Kretschy: Deutsches Arch. f. klin. Med., xviii, p. 527; Jahresbericht f. Thierchemie, 1876, vi, p. 173.

[3] Buchner: Deutsches Arch. f. klin. Med., xxix, p. 537; Jahresbericht f. Thierchemie, 1881, xi, p. 286.

the human stomach alcohol, wine, and beer all retarded digestion, though not so markedly as in artificial digestion. Bikfalvi,[1] in observations on dogs, obtained a retardation of digestion with even small quantities of alcohol. Beer and wine showed no favorable influence, the latter even retarding digestion when given in large quantities. Ogáta[2] states that beer, wine, and brandy retard gastric digestion noticeably. Schelhaas[3] observed that in the living stomach wine did not retard digestion so long as there was free HCl present; pathological conditions (carcinoma ventriculi) formed the only exceptions. In an extensive series of experiments, Gluzinski[4] distinguishes two phases occurring during digestion in the stomach in the presence of alcohol, (1) a retardation of proteid digestion, and (2) secretion of a very active, strongly acid gastric juice. Henczinski[5] found no bad effect on digestion following the use of beer. Blumenau[6] states that 25–50 per cent alcohol introduced into the healthy stomach induces a decrease in digestive action during the first two or three hours. Wolffhardt,[7] experimenting on a healthy man, concluded that 15–20 grams of absolute alcohol interfere with proteid digestion, while the effect of cognac varies with the period of digestion during which it is taken; he found that wines tend to promote digestion.

With reference to the motor functions of the stomach Lauder Brunton states that alcohol taken into this organ increases its movements as well as its secretory activity, and by mixing its contents more thoroughly with the gastric juice accelerates digestion.[8] Likewise Klemperer[9] states as a result of his experiments that the motor

[1] Bikfalvi: Jahresbericht f. Thierchemie, 1885, xv, p. 273.

[2] Ogáta: Jahresbericht f. Thierchemie, 1885, xv, p. 274; Arch. f. Hygiene, 1885, iii, p. 204.

[3] Schelhaas: Deutsches Arch. f. klin. Med., xxxvi, p. 427; Jahresbericht f. Thierchemie, 1885, xv, p. 271.

[4] Gluzinski: Deutsches Arch. f. klin. Med., 1886, xxxix, p. 405; Jahresbericht f. Thierchemie, 1886, xvi, p. 263.

[5] Henczinski: Dissertation, 1886. Quoted by Munk: Die Ernährung, p 327.

[6] Blumenau: Therapeutische Monatshefte, 1890, v, p. 504; Jahresbericht f. Thierchemie, 1891, xxi, p. 212.

[7] Wolffhardt: Münchn. med. Wochenschr., 1890, xxxvii, p. 608; Centralbl. f. med. Wissen., 1891, p. 47.

[8] Brunton: Disorders of digestion, 1886, p. 146.

[9] Klemperer: Zeitschr. f. klin. Med., 1890, xvii, Supp., p. 324; Centralbl. f. med. Wissen, 1891, p. 751.

functions are decidedly increased, as measured by the oil method, while Haan[1] has more recently advanced similar conclusions as the result of work by another method. Gluzinski,[2] however, notes that alcohol diminishes the mechanical action of the stomach in moderate degree.

In considering the selection of subjects for experiment in the direction indicated, preference has been given to dogs. The series of investigations on man above referred to are already extensive, and the difficulties of obtaining definite answers to specific questions by this method of experimentation are obvious. It is rarely possible or desirable to carry out a large number of determinations on any single individual, while it is likewise practically impossible to control the physiological condition of the individual, *i. e.*, diet, etc., over prolonged periods. The animals used in this research were large dogs of 21 and 25 kilos; gastric fistulæ were made, and a German silver cannula introduced into the fundus of the stomach.. In place of a cork, metal stoppers were devised to screw into the inner cannula tube by means of a small metallic key. The arrangement is shown in the diagram. The wounds healed perfectly, and the animals remained in good health during the entire period of investigation, covering several months. Irregularities of diet were avoided by feeding definite portions of prepared dog biscuit with water; this food was eagerly eaten, and sufficed to keep the dogs in physiological equilibrium.

The determinations of the acidity of the stomach contents were carried out according to the method of Topfer.[3] The gastric fluid was occasionally centrifugalized when food particles prevented pipetting off the fluid portion. Where only small quantities of fluid were available the titrations with phenolphthalien and dimethylamidoazobenzol were combined in the same 5 c.c. of fluid according to the recommendation of Einhorn.[4] Comparative experiments show that this modification gives the same values as the original method. Thus in one experiment: —

[1] Haan: Comptes rendus de la société de biologie, 1895, ii, p. 816.
[2] Gluzinski: *loc. cit.*
[3] Töpfer: Zeitschr. f. physiol. Chemie, 1894, xix, p. 104.
[4] Einhorn: New York medical journal, 1896, May 9, p. 603.

	Total acidity with *Phenolphthalein.*	Free HCl with *Dimethylamidoazobenzol.*
Töpfer method . . . . . (separate titrations)	1.55 c.c. $\frac{N}{10}$ NaOH = 0.112 per cent HCl.	1.0 c.c. $\frac{N}{10}$ NaOH = 0.072 per cent HCl.
Einhorn-Töpfer method . . (combined titration)	1.55 c.c. $\frac{N}{10}$ NaOH = 0.112 per cent HCl.	1.0 c.c. $\frac{N}{10}$ NaOH = 0.072 per cent HCl.

Our experience with Töpfer's method (or Einhorn's modification) leads us to agree with P. Häri [1] that in the absence of free HCl., *i. e.*, when no reaction is obtained with the dimethylamidoazobenzol reagent, the quantitative determinations of HCl by this method cease to be accurate, and under such conditions it cannot be employed. The occurrence of such conditions, however, is not frequent in the dog; we have observed the absence of free HCl (during digestion) in one animal under circumstances resembling those of acute gastric catarrh.[2] The food — dog biscuit — was largely undigested many hours after the meal, the acidity was high (0.55–0.594 per cent expressed as HCl), and the gastric contents possessed an odor strongly suggesting fatty acids. Lactic acid was found present (Uffelmann's test).

In view of the increased volume of fluid found in the stomach when alcohol is introduced into that organ after ligation of the pylorus, it was of interest to learn what results follow under normal conditions of the pylorus. For this purpose 20 to 25 per cent alcohol, slightly warmed, was introduced through the gastric cannula, and at the end of 30 minutes the gastric contents were discharged into a graduated vessel. Control experiments were made with distilled water, both fluids always being introduced into the empty stomach. This condition of the organ is shown by the lack of spontaneous flow when the cannula is opened, as well as by absence of free HCl. Flocks of mucus, alkaline to litmus, are usually present. The data obtained show no marked agreement, the fluid as a rule

[1] Häri, P.: Arch. f. Verdauungskrankh., ii. pp. 182, 332: Centralbl. f. Physiologie, 1896, x, p. 731.

[2] Cf. v. Jaksch: Klinische Diagnostik innerer Krankheiten, 4te Auflage, p. 200.

rapidly disappearing from the stomach. In 17 experiments with water, the *average* relative volume recovered from the stomach through the cannula at the end of the thirty minutes after introduction of quantities from 40–200 c.c. was about 30 per cent. Fourteen similar experiments with alcohol gave an average of 45 per cent. It is natural to ascribe the relatively greater volumes found in the stomach after the use of alcohol to an increased secretion of gastric juice occurring along with the rapid expulsion of fluid through the pylorus, and not to a retardation of the motor functions; for current statements assume increased motility of the stomach under the influence of alcohol,[1] while the experiments already reported justify the explanation given. Much emphasis cannot, however, be placed upon the averages given above, since the individual results vary widely among themselves, and no constant corresponding variations in acidity were observed, as in the experiments with ligated pylorus.

In the following series of experiments test meals were given, and the influence of alcohol and a considerable number of alcoholic beverages contrasted with that of water. Attention was directed to (1) variations in acidity and (2) time of digestion. Fifty grams of finely chopped lean meat were fed to the dog in each experiment, the stomach having been previously examined and found empty. Meat was chosen for the test meal because experience in this laboratory has shown that its composition, when it is obtained as described, does not vary much from time to time; and after a trial of mixed food, *e. g.* dog biscuit, it seemed more satisfactory to employ a simple diet in which proteid preponderated. Similar recommendation is made by v. Jaksch in considering test meals for the human subject.[2] Alcoholic fluids or water were introduced slightly warmed[3] into the stomach through the fistula, since dogs usually refuse to take the former by way of the mouth. At definite intervals of one-quarter to one-half hour, small quantities of gastric contents were permitted to flow out of the fistula. Total acidity (expressed as HCl), free and loosely combined HCl were determined by the method already described. The process of digestion in the stomach lasted, under the conditions described, about three hours, the average duration varying

[1] Cf. references p. 190.

[2] v. Jaksch: *loc. cit.*, p. 192.

[3] Cf. note 4, p. 176.

somewhat with the animal.[1] There was no very gradual diminution of undissolved meat particles noticeable until toward the end of this period, when the stomach very soon became empty. This corresponds with the observations of Kühne on man and the dog, in experiments with duodenal fistulæ.[2] This investigator found only a slight disappearance of contents from the stomach until near the end of the digestion period, when the great bulk of material, excepting larger pieces of food, was discharged at once through the pylorus. Richet arrived at similar conclusions in experiments on man.[3] We have usually observed a complete emptying of the stomach within a period of thirty minutes; the conclusion of this process is designated in the notes as the "end of gastric digestion." Protocols of experiments follow.

ANALYSES OF ALCOHOLIC BEVERAGES USED.

	Alcohol by vol. per cent.	Dry solids. per cent.		Alcohol by vol. per cent.	Dry solids. per cent.
Gin	51.0	0.29	Stout . . .	6.2	5.4
Whiskey . .	50.0	0.32	Claret . . .	5.2	3.2
Sherry . . .	21.75	4.7	Porter . . .	5.3	4.4
White Wine .	13.32	2.5	Beer . . .	4–5	7.0

DOG A. — Weight 25 kilos.

I. 9.25 A.M. **50 grams meat** (no water).

	ANALYSIS OF CONTENTS		
	Total acidity.	*Loosely combined HCl.*	*Free HCl.*
9.55	0.382	0.292	0.104
10.35	0.425	0.234	0.148
11.10	0.425	0.220	0.180
11.45	0.407	0.224	0.176
12.15	Stomach empty; end of gastric digestion.		

Time of digestion = **2 hours and 55 minutes.**

[1] In experiments on a man, with a similar meal, Jessen found the digestion time equalled 2 to 3 hours. Zeitschr. f. Biologie, 1883, xix, p. 149.

[2] KÜHNE: Lehrbuch der physiol. Chemie, 1868, p. 53.

[3] RICHET: Quoted in GAMGEE: Physiological chemistry, 1893, ii, p. 159.

II. 9.10 A.M. 50 grams meat + **50 c.c. water.**

	ANALYSIS OF CONTENTS		
	Total acidity.	*Loosely combined HCl.*	*Free HCl.*
9.30	0.241	0.144	0.093
10.00	0.295	0.169	0.108
10.20	0.367	0.216	0.115
10.40	0.439	0.288	0.144
11.30	Stomach empty; end of gastric digestion.		
	Time of digestion = **2 hours and 20 minutes.**		

III. 9.30 A.M. 50 grams meat + **100 c.c. water.**

	ANALYSIS OF CONTENTS		
	Total acidity.	*Loosely combined HCl.*	*Free HCl.*
10.00	0.299	0.173	0.080
10.30	0.475	0.230	0.122
11.00	0.518	0.230	0.173
11.15	0.497	0.202	0.241
11.35	0.494	0.191	0.202
11.50	0.479	0.205	0.195
12.10	0.382	0.194	0.187
12.30	Stomach empty; end of gastric digestion		
	Time of digestion = **3 hours.**		

IV. 2.10 P.M. 50 grams meat + **150 c.c. water.**

	ANALYSIS OF CONTENTS		
	Total acidity.	*Loosely combined HCl.*	*Free HCl.*
2.40	0.252	0.137	0.108
3.10	0.374	0.194	0.130
3.40	0.533	0.245	0.198
3.55	0.547	0.234	0.234
4.10	0.490	0.205	0.216
4.25	0.385	....	0.101
4.40	Stomach empty; end of gastric digestion.		
	Time of digestion = **2 hours and 30 minutes.**		

V. 9.05 A.M. 50 grams meat + **150 c.c. carbonated water.**

	ANALYSIS OF CONTENTS		
	Total acidity.	*Loosely combined HCl.*	*Free HCl.*
9.35	0.263	0.083	0.122
10.05	0.360	0.158	0.140
10.35	0.468	0.194	0.216
10.50	0.486	0.205	0.216
11.05	0.540	0.234	0.198
11.25	0.580	0.234	0.248
11.45	Stomach empty; end of gastric digestion.		
	Time of digestion = **2 hours and 40 minutes.**		

VI. 1.00 P.M. 50 grams meat + **100 c.c. 10 per cent alcohol.**

	ANALYSIS OF CONTENTS		
	Total acidity.	*Loosely combined HCl.*	*Free HCl.*
2.45	0.497	0.209	0.230
3.10	0.464	0.220	0.173
3.30	0.436	0.180	0.202
3.50	0.400	0.162	0.202
4.10	0.263	....	0.094

4.30 Stomach empty; end of gastric digestion.

Time of digestion = **3 hours and 30 minutes.**

VII. 2.30 P.M. 50 grams meat + **50 c.c. 20 per cent alcohol.**

	ANALYSIS OF CONTENTS		
	Total acidity.	*Loosely combined HCl.*	*Free HCl.*
3.00	0.313	0.118	0.090
3.30	0.374	0.187	0.176
4.00	0.439	0.194	0.151
4.30	0.515	0.205	0.184
5.00	0.407	0.144	0.248
5.30	0.264	....	0.155

5.30 Stomach nearly empty; end of gastric digestion.

Time of digestion = **3 hours.**

VIII. 12.45 P.M. 50 grams meat + **50 c.c. 20 per cent alcohol.**

	ANALYSIS OF CONTENTS		
	Total acidity.	*Loosely combined HCl.*	*Free HCl.*
2.30	0.439	0.213	0.158
2.50	0.457	0.191	0.205
3.10	0.493	0.205	0.227
3.30	0.364	0.129	0.187

3.50 Stomach practically empty; end of gastric digestion.

Time of digestion = **3 hours and 5 minutes.**

IX. 9.15 A.M. 50 grams meat + **50 c.c. 30 per cent alcohol.**

	ANALYSIS OF CONTENTS		
	Total acidity.	*Loosely combined HCl.*	*Free HCl.*
9.40	0.191	0.130	0.058
10.05	0.335	0.155	0.151
10.30	0.421	0.176	0.180
10.50	0.468	0.184	0.201
11.10	0.460	0.165	0.220
11.30	0.410	0.148	0.220
11.50	0.468	0.195	0.244
12.10	0.417	0.112	0.240
12.30	0.360	0.086	0.216

1.00 Stomach empty; end of gastric digestion.

Time of digestion = **3 hours and 45 minutes.**

X. 9.00 A.M. 50 grams meat + **150 c.c. Hochheimer.**

	ANALYSIS OF CONTENTS		
	Total acidity.	*Loosely combined HCl.*	*Free HCl.*
9.30	0.374	0.140	0.176
10.00	0.432	0.154	0.191
10.15	0.450	0.151	0.198
10.45	0.497	0.187	0.220
11.15	0.533	0.198	0.271
11.30	0.555	0.241	0.227
12.00	0.508	0.248	0.173
12.15	Stomach empty; end of gastric digestion.		

Time of digestion = **3 hours and 15 minutes.**

XI. 9.00 A.M. 50 grams meat + **50 c.c. whiskey** + **50 c.c. water.**

	ANALYSIS OF CONTENTS		
	Total acidity.	*Loosely combined HCl.*	*Free HCl*
9.30	0.252	0.101	0.119
10.00	0.392	0.176	0.176
10.30	0.403	0.151	0.191
11.00	Stomach empty; end of gastric digestion.		

Time of digestion = **2 hours.**

XII. 2.45 P.M. 50 grams meat + **50 c.c. whiskey** + **50 c.c. water.**

	ANALYSIS OF CONTENTS		
	Total acidity.	*Loosely combined HCl.*	*Free HCl.*
3.15	0.230	0.076	0.119
3.45	0.320	0.097	0.220
4.15	0.468	0.198	0.212
4.30	0.508	0.198	0.198
4.45	0.490	0.184	0.212
5.15	0.569	0.205	0.252
5.45	Stomach empty; end of gastric digestion.		

Time of digestion = **3 hours.**

XIII. 1.00 P.M. 50 grams meat + **50 c.c. gin** + **25 c.c. water.**

	ANALYSIS OF CONTENTS		
	Total acidity.	*Loosely combined HCl.*	*Free HCl.*
2.00	0.439	0.173	0.194
2.30	0.450	0.170	0.197
2.45	0.428	0.158	0.238
3.00	0.442	0.154	0.212
3.15	0.410	0.140	0.215
3.30	0.420	0.143	0.234
3.45	0.338	0.122	0.180
4.00	Stomach empty; end of gastric digestion.		

Time of digestion = **3 hours.**

XIV. 9.20 A.M. 50 grams meat + **50 c.c. brandy** + **25 c.c. water.**

	ANALYSIS OF CONTENTS		
	Total acidity.	*Loosely combined HCl.*	*Free HCl.*
9.50	0.237	0.159	0.065
10.20	0.368	0.201	0.133
10.50	0.465	0.230	0.205
11.20	0.533	0.267	0.194
11.40	0.468	....	0.158
12.00	Stomach empty; end of gastric digestion.		

Time of digestion = **2 hours and 40 minutes.**

XV. 2.50 P.M. 50 grams meat + **150 c.c. lager beer.**

	ANALYSIS OF CONTENTS		
	Total acidity.	*Loosely combined HCl.*	*Free HCl.*
3.20	0.259	0.112	0.115
3.50	0.410	0.205	0.148
4.20	0.518	0.245	0.184
4.35	0.572	0.248	0.230
4.50	0.569	0.252	0.208
5.05	0.547	0.220	0.238
5.20	0.508	0.162	0.211
5.35	0.475	0.162	0.238
5.50	0.413	0.115	0.241
6.05	Stomach empty; end of gastric digestion.		

Time of digestion = **3 hours and 15 minutes.**

XVI. 9.40 A.M. 50 grams meat + **150 c.c. stout.**

	ANALYSIS OF CONTENTS		
	Total acidity.	*Loosely combined HCl.*	*Free HCl.*
10.10	0.364	0.140	0.187
10.40	0.446	0.166	0.180
11.10	0.555	0.220	0.295
11.40	0.616	0.212	0.302
12.10	0.580	0.266	0.247
12.40	Stomach empty; end of gastric digestion.		

Time of digestion = **3 hours.**

XVII *a.* 9.15 A.M. 50 grams meat + **150 c.c. beer.**

	ANALYSIS OF CONTENTS		
	Total acidity.	*Loosely combined HCl.*	*Free HCl.*
9.45	0.248	0.151	0.082
10.15	0.367	0.201	0.123
10.45	0.457	0.238	0.137
11.20	0.526	0.266	0.209
11.40	0.511	0.213	0.223
12.15	0.465	0.216	0.176
12.30	Stomach empty; end of gastric digestion.		

Time of digestion = **3 hours and 15 minutes.**

XVII β. 3.00 P.M. 50 grams meat + **150 c.c. water.**

	ANALYSIS OF CONTENTS		
	Total acidity.	*Loosely combined HCl.*	*Free HCl.*
3.30	0.227	0.130	0.080
4.00	0.400	0.209	0.129
4.30	0.522	0.274	0.158
5.00	0.583	0.310	0.195
5.15	0.583	0.302	0.205
5.30	0.446	0.209	0.184
5.45	0.569	0.298	0.127
6.00	Stomach empty; end of gastric digestion.		
	Time of digestion = **3 hours**.		

XVIII α. 8.30 A.M. 50 grams meat + **50 c.c. water.**

	ANALYSIS OF CONTENTS		
	Total acidity.	*Loosely combined HCl.*	*Free HCl.*
9.00	0.371	0.227	0.126
9.30	0.443	0.274	0.144
10.00	0.518	0.252	0.234
10.30	0.569	0.263	0.252
11.00	Stomach empty; end of gastric digestion.		
	Time of digestion = **2 hours and 30 minutes.**		

XVIII β. 2.10 P.M. 50 grams meat + **100 c.c. 30 per cent alcohol.**

	ANALYSIS OF CONTENTS		
	Total acidity.	*Loosely combined HCl.*	*Free HCl.*
2.40	0.234	0.112	0.101
3.10	0.352	0.165	0.137
3.40	0.490	0.209	0.162
4.10	0.550	0.263	0.191
4.40	0.550	0.245	0.201
5.10	Stomach empty; end of gastric digestion.		
	Time of digestion = **3 hours**.		

XIX α. 9.00 A.M. 50 grams meat + **100 c.c. water.**

	ANALYSIS OF CONTENTS		
	Total acidity.	*Loosely combined HCl.*	*Free HCl.*
9.30	0.324	0.165	0.137
10.00	0.378	0.198	0.144
10.30	0.494	0.259	0.169
11.00	0.487	0.220	0.188
11.15	0.457	0.205	0.131
11.30	Stomach empty; end of gastric digestion.		
	Time of digestion = **2 hours and 30 minutes.**		

XIX β. 2.30 P.M. 50 grams meat + **150 c.c. lager beer.**

	ANALYSIS OF CONTENTS		
	Total acidity.	*Loosely combined HCl.*	*Free HCl.*
3.00	0.260	0.119	0.137
3.30	0.378	0.201	0.137
4.00	0.465	0.191	0.188
4.30	0.533	0.223	0.248
4.45	0.562	0.233	0.306
5.10	0.465	0.223	0.176
5.30	Stomach empty; end of gastric digestion.		
	Time of digestion = **3 hours.**		

XX α. 9.15 A.M. 50 grams meat + **75 c.c. sherry** + **25 c.c. water.**

	ANALYSIS OF CONTENTS		
	Total acidity.	*Loosely combined HCl.*	*Free HCl.*
9.45	0.295	0.108	0.155
10.15	0.331	0.101	0.173
10.45	0.367	0.133	0.187
11.15	0.418	0.158	0.212
11.30	0.436	0.169	0.216
11.45	0.490	0.191	0.248
12.00	Stomach empty; end of gastric digestion.		
	Time of digestion = **2 hours and 45 minutes.**		

XX β. 2.30 P.M. 50 grams meat + **150 c.c. carbonated water.**

	ANALYSIS OF CONTENTS		
	Total acidity.	*Loosely combined HCl.*	*Free HCl.*
3.00	0.238	0.043	0.126
3.30	0.360	0.130	0.176
4.00	0.432	0.187	0.169
4.30	0.533	....	0.169
4.45	Stomach empty; end of gastric digestion.		
	Time of digestion = **2 hours and 15 minutes.**		

Dog B. — Weight 21 kilos.

I. 1.45 P.M. **50 grams meat** (no water).

	ANALYSIS OF CONTENTS		
	Total acidity.	*Loosely combined HCl.*	*Free HCl.*
2.15	0.353	0.191	0.118
2.40	0.443	0.222	0.180
3.00	0.511	0.227	0.198
3.20	0.525	0.227	0.280
3.45	0.572	0.260	0.209
4.15	0.568	0.349	0.195
4.45	Stomach empty; end of gastric digestion.		
	Time of digestion = **3 hours.**		

II. 9.15 A.M. 50 grams meat + **50 c.c. water**.

	ANALYSIS OF CONTENTS		
	Total acidity.	*Loosely combined HCl.*	*Free HCl.*
9.50	0.302	0.220	0.082
10.15	0.432	0.223	0.144
10.45	0.472	0.201	0.252
11.15	0.472	0.144	0.288
11.35	0.484	0.155	0.270
11.55	0.453	0.144	0.306
12.15	0.407	0.100	0.241
12.30	0.400	0.133	0.234
12.45	0.306	....	0.216

End of gastric digestion.
Time of digestion = **3 hours and 30 minutes**.

III. 9.15 A.M. 50 grams meat + **50 c.c. 20 per cent alcohol** + **water.**

	ANALYSIS OF CONTENTS		
	Total acidity.	*Loosely combined HCl.*	*Free HCl.*
9.50	0.136	0.086	0.036
10.15	0.285	0.108	0.144
10.45	0.479	0.173	0.244
11.15	0.472	0.177	0.252
11.35	0.518	0.237	0.252
11.55	0.486	....	0.209
12.15	0.421	....	0.213
12.30	Stomach empty: end of gastric digestion.		

Time of digestion = **3 hours and 15 minutes**.

IV. 8.50 A.M. 50 grams meat + **100 c.c. 30 per cent alcohol.**

	ANALYSIS OF CONTENTS		
	Total acidity.	*Loosely combined HCl.*	*Free HCl.*
9.20	0.324	....	0.144
9.50	0.493	....	0.072
10.20	0.641	....	0.100
10.50	0.547	0.338	0.166
11.20	0.588	....	0.206
11.50	0.544	....	0.230
12.20	....	....	present.
12.30	End of gastric digestion.		

Time of digestion = **3 hours and 40 minutes**.

V. 2.45 P.M. 50 grams meat + **75 c.c. claret**.

	ANALYSIS OF CONTENTS		
	Total acidity.	*Loosely combined HCl.*	*Free HCl.*
3.15	0.396	0.155	0.216
3.45	0.450	0.238	0.158
4.15	0.576	....	0.209
4.45	End of gastric digestion.		

Time of digestion = **2 hours**.

VI α. 9.15 A.M. 50 grams meat + **150 c.c. beer.**

	ANALYSIS OF CONTENTS		
	Total acidity.	*Loosely combined HCl.*	*Free HCl.*
9.45	0.273	0.144	0.104
10.15	0.367	0.187	0.155
10.45	0.464	0.223	0.194
11.15	0.616	0.345	0.256
11.45	0.501	0.238	0.170
12.15	0.508	....	0.151
12.30	0.533	....	0.187
12.45	0.468	....	0.158
1.00	End of gastric digestion.		
	Time of digestion = **3 hours and 45 minutes.**		

VI β. 1.00 P.M. 50 grams meat + **150 c.c. water.**

	ANALYSIS OF CONTENTS		
	Total acidity.	*Loosely combined HCl.*	*Free HCl.*
2.00	0.620	0.282	0.201
2.30	0.590	0.266	0.234
3.00	0.666	0.392	0.224
3.30	0.627	....	0.206
4.00	....	....	trace.
4.15	End of gastric digestion.		
	Time of digestion = **3 hours and 15 minutes.**		

In the accompanying table the "time of digestion" of the experiments preceding is given in hours. The experiments marked *α* and *β* are strictly comparable, as reference to the protocols will show that they were carried out in succession on the same day.

From these results it is apparent that the time of digestion in the stomach for the proteid test meal employed is not greatly varied under the influence of alcohol. The results obtained suggest, however, a tendency toward prolongation of the period during which the meat remains in the stomach when alcoholic fluids are present. This tendency is most noticeable in the case of Dog A, and particularly in those experiments which immediately succeed each other on the same day and are therefore strictly comparable. The differences are too small, however, to have any great significance. Retardation is perhaps more marked with the malted beverages, and is apparently out of proportion to the alcohol present. With reference to the changes in the acidity of the stomach contents a large number of observations disclose no specific differences in the various digestions. The varia-

TABLE OF TIME OF DIGESTION (IN HOURS).

Dog A.					Dog B.			
No.	Water.	Alcohol.	Weak alcoholic beverages.	Strong alcoholic beverages.	No.	Water.	Alcohol.	Weak alcoholic beverages.
	h. m.	h. m.	h. m.	h. m.		h. m.	h. m.	h. m.
XVII α	....	....	3 15	....				
XVII β	3	....	....	....	I	3	....	....
I	2 55	....	....	....	II	3 30	....	....
II	2 20	....	....	....	III	....	3 15	....
VII	....	3	....	....	IV	....	3 40	....
VI	....	3 30	....	....	V	....	....	2
VIII	....	3 05	....	....	VI α	....	....	3 45
IX	....	3 45	....	....	VI β	3 15	....	....
XVIII α	2 30	....	....	....		....	....	....
XVIII β	....	3	....	....		....	....	....
XIV	....	....	....	2 40		....	....	....
XV	....	....	3 15	....		....	....	....
XIX α	2 30	....	....	....		....	....	....
XIX β	....	....	3	....		....	....	....
XVI	....	....	3	....		....	....	....
IV	2 30	....	....	....		....	....	....
X	....	....	3 15	....		....	....	....
III	3	....	....	....		....	....	....
XIII	....	....	....	3		....	....	....
XX α	....	....	....	2 45		....	....	....
XX β	2 15	....	....	....		....	....	....
V	2 40	....	....	....		....	....	....
XI	....	....	....	2		....	....	....
XII	....	....	....	3		....	....	....
Average.	2 40	3 20	3 10	2 40		3 15	3 30	2 52

tions are common to all the experiments. They include a gradual rise in total acidity during approximately the first two hours of diges-

tion, followed by a gradual decrease until the stomach becomes empty; at this point free HCl is absent. The combined HCl increases with the progress of digestion, the products of proteolysis combining with relatively larger quantities of free acid.[1] Since the secretion of acid is continually progressing in the stomach, the percentage of free HCl increases gradually in the course of the digestion, likewise decreasing rapidly toward the end of this process. In agreement with our previous statements relative to the rather sudden discharge of the gastric contents into the intestine (p. 194), an abrupt decline in acidity toward the end of the digestion period was frequently observed. Evidence of an "after period" of secretion was not obtained.[2]

Disappearance of Alcohol from the Stomach.

It has long been known that alcohol disappears rapidly from the alimentary canal, and even so early as 1847 Bouchardat and Sandras stated that the absorption takes place from the stomach especially.[3] More recent and conclusive experiments in which the pylorus has been artificially closed, have demonstrated with certainty that alcohol, in distinction from water, is readily absorbed from the stomach.[4] Furthermore, many substances like sugar, peptone, etc., are readily absorbed from the stomach in the presence of alcohol, while their absorption from the intestine is likewise accelerated by this substance.[5] Thus an ordinary dose of chloral hydrate introduced in watery solution into a stomach with ligated pylorus fails to bring about narcosis;[6] if, however, a quantity of alcohol too small of itself to produce any pharmacological action be present, narcosis follows, just as when the open pylorus permits the intestine to participate in the absorption.

The complete disappearance of alcohol from the stomach has been observed by us in a large number of experiments in which the pylorus

[1] Cf. Chittenden: Digestive proteolysis, 1894, pp. 53 *seq.*

[2] Cf. Gluzinski: Jahresbericht f. Thierchemie, 1886, xvi, p. 264.

[3] Bouchardat and Sandras: Annales de chimie et de physique, 1847, xxi. 3 Série, p. 456.

[4] Cf. for example, Tappeiner: Zeitschr. f. Biologie, 1881, xvi, p. 497; Brandl: *ibid.*, 1892, xxix, p. 277; v. Mering: Jahresbericht f. Thierchemie, 1893, xxiii, p. 293.

[5] Cf. for example, J. v. Scanzoni: Zeitschr. f. Biologie, 1896, xxxiii, p. 462.

[6] Cf. also experiments with strychnine. Meltzer: Journ. of exper. medicine, 1896, i, p. 529.

was ligated. The following results tabulated from the experiments on secretion (pp. 179–186), demonstrate this statement: —

TABLE SHOWING ABSORPTION OF ALCOHOL FROM STOMACH.

No.	Weight of dog. Kilos.	Duration of experiment.	Volume of fluid introduced. ccm.	Content of alcohol. Per cent by vol.	Alcohol found at end of experiment. grams.
		h. m.			
VII	23.0	3 30	200 (alcohol)	37.5	4
VIII	21.0	3 00	200 (")	37.5	4.5
IX	8.0	3 56	100 (")	5.0	0
X	7.3	3 45	110 (")	4.8	0
XIII	10.7	3 55	75 (sherry)	21.0	0
XIV	18.5	3 45	150 (whiskey)	16.0	0
XV	8.0	3 45	125 (wine)	13.3	0
XVI	25.0	3 00	135 (")	13.3	0
XVII	12.3	4 00	125 (claret)	5.15	0
XVIII	10.2	3 55	100 (beer)	4.5	0
XX	14.0	3 45	150 (porter)	3.75	0
XXI	8.5	3 55	125 (beer)	4.7	0

The rapid discharge of watery or alcoholic fluids from the stomach through the pylorus has already been referred to on p. 193. The results are in harmony with those obtained by v. Mering on dogs with duodenal fistulæ.[1] In his experiments, for example, 500 c.c. being administered to a large dog, 490 c.c. were expelled through the pylorus in twenty minutes. The rapidity of expulsion was found to depend on the state of repletion of the small intestine, — an observation in accord with the retarded evacuation of the stomach seen when food is given along with fluids. v. Mering further observed that when water holding CO_2 in solution enters the stomach, the gas is readily absorbed;[2] alcohol is likewise absorbed, as J. Miller has recently verified for the human stomach.[3] Ogáta[4] found that of 6.5–8.8 grams

[1] v. Mering: Quoted in Gamgee: Physiological chemistry, 1893, ii, pp. 441 *seq.*

[2] Cf. also Experiment V., p. 178.

[3] Miller, J.: Arch. f. Verdauungskrankh., i, p. 233. Jahresbericht f. Thierchemie, 1895, xxv, p. 293.

[4] Ogáta: Jahresbericht f. Thierchemie, 1885, xv, p. 274.

of alcohol introduced into the stomach in wine or beer, 80–90 per cent disappeared within half an hour. In the presence of soluble products in the stomach, an excretion of water by that organ is said to result in proportion to the amount of substance absorbed, — an idea akin to the one suggested in explanation of the relatively larger quantities of fluid found in the unligated stomach soon after introduction of alcohol, as compared with water. The experiments which we have made verify the statements of the investigators mentioned, as the following data selected from protocols indicate: —

Data showing disappearance of alcohol from unligated stomach.

I. Dog, with gastric fistula.

a. 3.45 p. m. Introduced *50 c.c. 20 per cent alcohol* into stomach.
4.15 " Removed gastric contents = 40 *c.c. No alcohol found.*

b. 3.15 " Introduced *40 c.c. 25 per cent alcohol.*
3.45 " Removed gastric contents = *20 c.c. No alcohol found.*

c. 2.40 " Introduced *125 c.c. 20 per cent alcohol.*
3.10 " Removed a portion of gastric contents. Free HCl = 0.072 per cent. *Small amount of alcohol present.*

II. Dog of 18 kilos, employed in a salivary experiment. In the course of the latter the animal received at intervals *45 c.c. absolute alcohol* diluted with water. Two hours after last portion was given the stomach contents (200 c.c.) were removed. They contained *1.1 grms. alcohol.*

III. Dog of 18 kilos. Salivary experiment. At intervals were given *70 c.c. absolute alcohol* diluted with water. One and one-third hours after last portion (40 c.c.) was given the stomach contents (350 c.c.) contained *9.4 grms. alcohol.*

IV. Dog of 14 kilos. Salivary experiment. *140 c.c. absolute alcohol* diluted with water were given in three portions. Three-fourths of an hour after the last portion (50 c.c.), the stomach contents (450 c.c.) contained *24.6 grms. alcohol.*

V. Dog of 10 kilos. Salivary experiment. *120 c.c. whiskey*, containing 50 per cent of alcohol, were given in two portions. Four and one-half hours after the last portion (60 c.c.) the stomach contents (170 c.c.). contained *2.7 grms. alcohol.*

VI. Dog. Salivary experiment. *135 c.c. brandy*, containing about 50 per cent of alcohol, were given in two portions. Two hours after last portion (75 c.c.), the stomach contents (240 c.c.) contained *8.8 grms. alcohol.*

VII. Dog of 10 kilos. Salivary experiment. *350 c.c. wine* containing 5.15 per cent alcohol were given in two portions. One and one-half hours after last portion (200 c.c.), the stomach contents (190 c.c.) contained *5.5 grms. alcohol.*

It is of interest to note that the large volumes of fluid (170–450 c.c.) found in the stomach in Experiments II.–VII. correspond with the data already presented with reference to the increased secretion of gastric juice due to alcohol and alcoholic beverages.

Summary.

Some of the more important conclusions to be drawn from the results of the experiments reported in the preceding pages may be advantageously summarized here.

Upon the secretion of saliva, the presence of strong alcohol or an alcoholic beverage in the mouth has a direct stimulating effect leading to a sudden increase in the flow of saliva. This acceleration of secretion, however, is of brief duration. The stimulating effect is manifested not only by an increase in the volume of the secretion, but also by an increase in both organic and inorganic constituents. The effect produced is in no sense peculiar to alcohol, but is common to many so-called stimulants, such as dilute acid (vinegar), ether-vapor, etc. Indeed, the effect is precisely analogous to that induced by an increase in intensity of stimulation, when the salivary glands are electrically excited through their nerves.

As to the possibility of alcoholic fluids absorbed from the stomach giving rise to an indirect stimulation of salivary secretion, or exercising any appreciable influence upon the composition of the secretion, our results give a negative answer. Thus, alcoholic fluids introduced directly into the stomach (of dogs) by injection through the stomach wall, thus doing away with any local action in the mouth, produce no appreciable effect upon the rate of secretion, as induced by a constant external stimulus, of either submaxillary or sublingual saliva. Even doses of alcohol sufficient to produce prolonged narcosis when introduced in this way fail to check the flow of saliva. There is likewise no specific influence exerted on the composition of the secretion. Hence, so far as our results go, alcohol and alcoholic fluids are without any specific effect upon the secretion of saliva, except to produce a transitory stimulation of secretion while in the mouth cavity.

Upon gastric secretion, alcohol and alcoholic fluids have a marked effect, increasing very greatly both the flow of gastric juice and also

its content of acid and total solids. Further, this action is exerted not only by the presence of alcoholic fluids in the stomach, but also indirectly through the influence of alcohol absorbed from the intestine. Thus, ordinary ethyl alcohol introduced into the empty stomachs of dogs, with the duodenum ligated, shows a marked stimulating action upon gastric secretion — as compared with the action of water under like conditions — increasing not only the volume of gastric juice very greatly, but also its acidity, content of solid matter, etc. Moreover, alcohol absorbed from the intestine, the latter being entirely shut off from the stomach, may likewise cause stimulation of the gastric glands, with a marked increase in the rate of secretion, etc. Whiskey, brandy, sherry, claret, beer, and porter all agree in producing stimulation of gastric secretion. Further, as already stated, the gastric juice secreted under alcoholic stimulation is more acid, contains more solid matter and more combined hydrochloric acid than the ordinary secretion. It is likewise strongly proteolytic.

If these results are considered in connection with our previous observations upon the influence of alcohol and alcoholic drinks upon the purely chemical processes of gastric digestion, it is seen that side by side with the greater or lesser retardation of digestive proteolysis caused by alcoholic beverages there occurs an increased flow of gastric juice rich in acid and of unquestionable digestive power. The two effects may thus normally counterbalance each other, though it is evident that modifying conditions may readily retard or stimulate the processes in the stomach according to circumstances. Foremost among the latter is the rapid disappearance of alcohol from the alimentary canal.

Since any influence exerted by alcohol or alcoholic beverages upon the solvent or digestive power of the gastric juice in the stomach must depend upon the presence of alcohol in the stomach contents, it follows that the tendency toward rapid removal of the alcohol from the alimentary tract by absorption must necessarily diminish correspondingly the extent of the retardation of gastric digestion which the presence of alcohol in the stomach may occasion. Since, however, the stimulation of gastric secretion induced by alcohol is brought about not only by the direct action of alcohol in the stomach, but also by the indirect action of alcohol absorbed from the intestine, it follows that possible inhibition of the digestive action of the gastric juice would probably be of shorter duration than the stimulation of secretion, and that consequently in the body alcoholic fluids would

hardly lead to any retardation of gastric digestion. This point has been very carefully and thoroughly tested by numerous experiments on healthy dogs with gastric fistulæ, using proteid test meals, with the result that certainly in the stomach of dogs digestion is not retarded in any pronounced degree under the influence of alcohol or alcoholic fluids. Of hastened digestion, the results obtained give little or no suggestion, and we must therefore conclude that the two diverse factors above referred to more or less counterbalance each other so that gastric digestion in the broadest sense of the term is not markedly varied under the influence of alcohol or alcoholic fluids. This conclusion, it may be mentioned, stands in perfect harmony with the results of the investigations of Zuntz and Magnus-Levy regarding the influence of alcohol (beer) on the digestibility and utilization of food in the body. These investigators found by a series of metabolic experiments on men with diets largely made up of milk and bread, and on individuals accustomed and unaccustomed to the use of alcoholic beverages, that the latter did not in any way diminish the utilization of the food by the body.[1]

Especially worthy of note is the rapid disappearance of alcohol from the stomach and alimentary tract when alcoholic fluids are taken. As our results show, the introduction of even 200 c.c. of 37 per cent alcohol into the stomach of a dog with the duodenum ligated at the pylorus may be followed by the nearly complete disappearance of the alcohol in 3–3½ hours by absorption through the stomach walls into the blood. With the outlet from the stomach into the intestine open, the rate of absorption of alcohol is greatly increased. We may well believe, as stated by Ogáta, that when 6–8 grams of alcohol are taken into the stomach in the form of wine or beer that 80–90 per cent of the alcohol will disappear from the alimentary tract inside of half an hour. Indeed, our own experiments on dogs with gastric fistulæ lead to this conclusion. Thus, in one experiment 50 c.c. of 20 per cent alcohol were introduced into the stomach, and on withdrawing the stomach-contents half an hour later no alcohol whatever was found in the 40 c.c. of fluid obtained. In view of this rapid disappearance of alcohol from the alimentary tract it is plain that alcoholic fluids cannot have much, if any, direct influence upon the secretion of either pancreatic or intestinal juice.

[1] Zuntz and Magnus-Levy: Archiv f. d. ges. Physiol., 1891, xlix. p. 438; Magnus-Levy: *ibid.*, 1893, liii, p. 544.

ON THE SIMILARITY OF STRUCTURAL CHANGES PRODUCED BY LACK OF OXYGEN AND CERTAIN POISONS.

By SIDNEY P. BUDGETT.

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WE know through the experiments of Spallanzani, Pflüger,[1] Bunge,[2] etc., that life phenomena may persist for a comparatively long period in the absence of oxygen, and that at the same time, as has been shown by Liebig and Hermann, a considerable amount of work may be done; but in the face of these facts we are at a loss to understand those cases in which asphyxia so quickly results in death.

Loeb[3] has suggested that when a function fails through lack of oxygen, the failure may in some cases be due to an alteration of the molecular conditions upon which depends the conversion of chemical energy into the particular form of energy set free in the physiological process concerned. In support of this view he describes the behavior of Ctenolabrus eggs when they are deprived of oxygen: eggs newly fertilized fail to divide, while those in which segmentation has reached the four-cell stage show a solution of the dividing surface layers, or cell membranes, and a return to a unicellular form. He points out that circumstances which lead to a breakdown of existing membranes would naturally prevent the formation of new ones, and calls attention to the probability that failure to divide depends rather upon the impossibility of membrane formation than upon lack of energy. In favor of this conclusion he mentions the fact that the eggs of a closely related fish, Fundulus, the cell membranes of which undergo no solution in an oxygen vacuum, may continue to divide for more than twelve hours.

He observed a similar difference in the two species, in the behavior of the embryonic heart, when its supply of oxygen was gradually diminished. In Ctenolabrus the heart was brought to a standstill too suddenly to allow the assumption that lack of energy was the

[1] PFLÜGER: Archiv f. d. ges. Physiol., 1875, x, p. 25.

[2] BUNGE: Zeitschr. f. physiol. Chemie, 1874, viii, p. 48.

[3] LOEB: Archiv f. d. ges. Physiol., 1895, lxii, p. 249.

cause, and he supposes that here also there may occur molecular and finally structural changes similar to those seen in the first stages of segmentation, and that these may prevent the conversion of chemical into mechanical energy. The heart of Fundulus, on the other hand, beat with increasing slowness until it reached a minimum rate, at which it continued as long as ten hours.

Loeb thinks it possible that the structural changes which occur in the absence of oxygen are the result of the action of abnormal compounds formed under these circumstances, and bases this supposition upon the fact that the products of metabolism are different when no oxygen is present, as had been shown by Araki,[1] who found lactic acid and sugar in the urine of dyspnœic animals.

Subsequently Broca and Richet,[2] in their study of the anærobic contraction of muscle, arrived at the conclusion that the injurious effects of lack of oxygen which they observed were due to the toxic action of unoxidized waste products.

At Professor Loeb's suggestion, I have subjected Paramœcia and other protozoa to the action of various poisons in order to determine whether any of these would produce structural changes similar to those appearing in an oxygen vacuum. With a number of the reagents used such was found to be the case.

Paramœcium aurelium. — The structural changes caused by lack of oxygen have been described by Loeb and Hardesty;[3] but their experiments have been repeated in the present series for the sake of comparison. A few drops of Paramœcium culture were enclosed in an Engelmann gas chamber, through which was passed hydrogen that had been washed in potassium hydrate solution, potassium permanganate solution, and water.

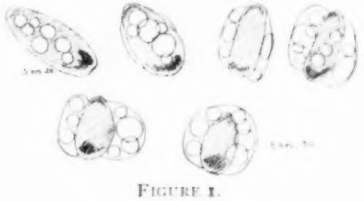

FIGURE 1.

After the stream of hydrogen has been passing for several hours, the time varying with the temperature of the room, the animals begin to swim more slowly, they absorb water, the contractile vacuoles increase in size, or several additional vacuoles may appear, and they sink to the bottom. One or more clear vesicles now protrude from the surface of the animal,

[1] ARAKI: Zeitschr. f. physiol. Chemie, 1891, xv, p. 325.

[2] RICHET: Archives de physiologie, 1876, viii, p. 829.

[3] LOEB and HARDESTY: Archiv f. d. ges. Physiol., 1895, lxi, p. 583.

and into these some of the vacuoles usually escape; the vesicles finally burst, and the cell contents are extruded (Fig. 1).

If a drop of a 0.1 per cent solution of potassium cyanide be added to several drops of Paramœcium culture, there result structural changes which are apparently exactly similar to those described above, but they occur immediately (Fig. 2). If the solution be more concentrated, for instance 0.5 per cent, the bursting often occurs at once, without a preceding marked change of form.

FIGURE 2.

Amœba. — When deprived of oxygen, Amœba becomes vacuolated and tends to assume a spherical form (Fig. 3). A dilute solution of antipyrine produces the same effects (Fig. 4). The same is true of digitaline and potassium cyanide solutions.

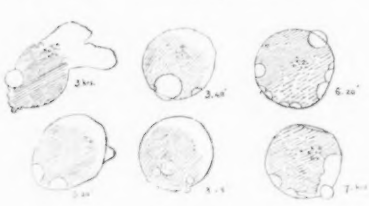

FIGURE 3.

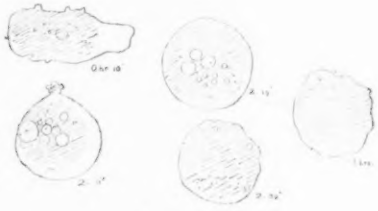

FIGURE 4.

Oxytricha (?) — After being subjected for several hours to a stream of hydrogen, this form begins to take up water, and becomes less flattened, the contractile vacuole being much distended. Like Paramœcia, it becomes sluggish and gradually sinks, the vacuole contracts, and it remains almost motionless at the bottom. Suddenly at or near one pole, usually the oral, the surface loses its smooth contour, the surface layer breaks down into small globules, and the change progresses toward the opposite pole, the protoplasm meanwhile becoming detached in spherical masses large and small; these quickly dissolve, and nothing is left of them but scattered granules. As the nuclei pass out they become spherical. The cilia usually, but not invariably, cease moving before the dissolution of the cell wall begins (Fig. 5).

FIGURE 5.

The addition of a dilute solution of potassium cyanide (Fig. 6) or pilocarpine to a culture of oxytricha causes similar changes in structure.

The same changes follow exposure to the action of veratrine (Fig. 7), sulphate of morphia, sulphate of quinine, antipyrine, nicotine, potassium or sodium hydrate, in sufficiently strong solutions, but the cilia usually continue to move until just before that portion of the cell wall upon which they are situated breaks down.

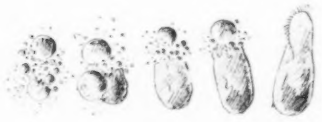

FIGURE 6.

FIGURE 7.

Under the influence of physostigmine (Fig. 8), atropine, or sulphate of strychnia, the aboral pole usually breaks down first, otherwise the visible results are the same.

FIGURE 8.

Jennings[1] has observed that "Paramœcia are not appreciably harmed" by placing them in distilled water. This rather surprising fact must depend upon their offering a great resistance to the entrance of water. The absorption of water, which forms such an important feature in the effects of lack of oxygen, and of poisoning, shows a reduction of this resistance, but probably depends also upon another factor, as is indicated by the following observation (Fig. 9).

FIGURE 9.

Figure 9 represents the changes shown by a sympathetic nerve cell from the Frog, when exposed to a five per cent solution of potassium cyanide. The drawings are from a series of twelve, which were made with a camera lucida in rapid succession. The osmotic pressure of the potassium cyanide solution being above that of the cell, water

[1] JENNINGS: Journal of physiology, 1897, xxi, p. 272.

at first passes out, and the cell shrinks rapidly. The subsequent swelling is probably due to extensive splitting up of molecules within the cell under the influence of the poison, and consequent rise of the intracellular osmotic pressure and absorption of water.

Conclusions.

The visible changes of structure shown by some protozoa when deprived of oxygen may be exactly reproduced by treatment with certain poisons. This indicates that either these poisons prevent oxidation or that lack of oxygen produces toxic substances.

Potassium cyanide, and perhaps other poisons, not only reduce the resistance normally shown by the Paramœcium to the entrance of water, but lead to the taking up of water probably by hastening the molecular breakdown and so increasing the osmotic pressure within the cell.

I desire to acknowledge the kind direction of Professor Loeb.

THE EFFECT OF DISTENTION OF THE VENTRICLE ON THE FLOW OF BLOOD THROUGH THE WALLS OF THE HEART.

By IDA H. HYDE, PH. D.

[*From the Laboratory of Physiology in the Harvard Medical School.*]

IN the course of the experiments "On the relation of the volume of the coronary circulation to the frequency and force of the ventricular contraction in the isolated heart of the cat," made in this Laboratory in 1896,[1] it was incidentally observed that distention of the left side of the isolated heart lessened the volume of the circulation through the coronary vessels, notwithstanding the maintenance of a constant pressure in the aorta. As distention of the heart is a state frequently observed in mountain climbers, athletes, hod-carriers, and many other classes, being indeed almost inseparable from violent, prolonged muscular efforts, and as it constitutes, in its severer forms, a disease of great clinical interest, a systematic pursuit of the clew which Magrath and Kennedy gave is well worth making.

The problem seemed a simple one. The coronary vessels of the isolated heart of the cat should be supplied with defibrinated cat's blood by maintaining a uniform blood-pressure in the aorta, and the outflow from the coronary veins, or, in other words, the volume of the coronary circulation, should be recorded by a suitable apparatus. The effect of the artificial distention of the left side of the heart upon this outflow would then be visible.

The apparatus for accomplishing these ends consisted of a reservoir in which the defibrinated blood could be kept at the temperature of the body; a pressure flask, by which the blood could be driven from the feeding reservoir into the aorta; a mercury manometer, for recording the blood-pressure in the aorta, so that the experimenter could be sure that the driving force remained the same throughout the observation; a membrane manometer, of the Hurthle type, for recording the changes of pressure in the left ventricle; a Mariotte flask, by which the intraventricular pressure could be raised to any height desired; a drop counter, for registering the

[1] MAGRATH, J. B., and H. KENNEDY: Journal of exper. medicine, 1897, ii, p. 13.

volume of the coronary circulation; and, finally, a kymograph, with a time recorder.

At the beginning of the experiment, two cats were anæsthetized with ether, tracheotomized, and bled from the left carotid artery. The blood was defibrinated, filtered through glass wool, and placed in the blood reservoirs to warm. Meanwhile, the front wall of the thorax of the second of the two cats was removed, exposing the heart and great vessels; the venæ cavæ and right vena azygos were ligated; cannulas were placed in the aorta, the left subclavian, pul-

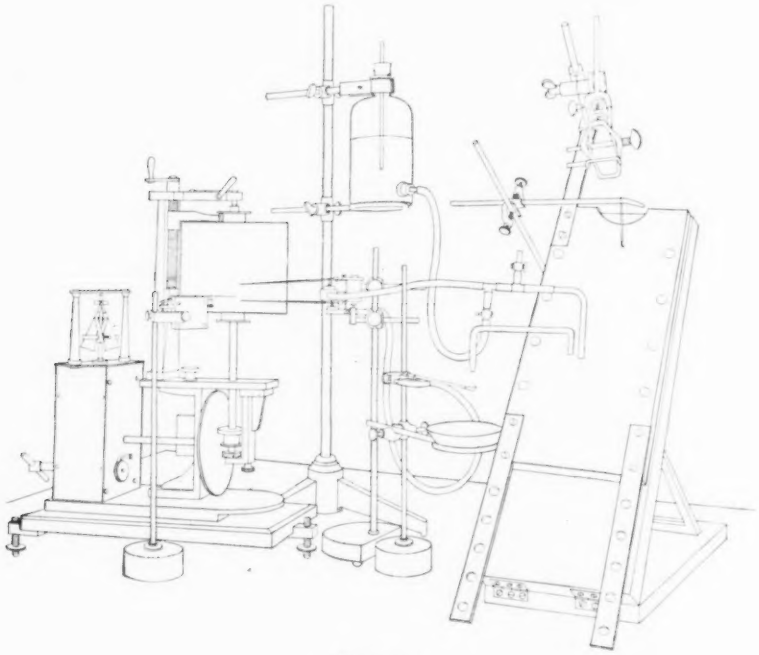

FIGURE 1.

monary, and innominate arteries; and a tube was passed into the left ventricle through the auricular appendix. After washing out the aorta with normal saline solution to remove blood clots and air, the cat, upon its Czermak board, was placed in the inclined stand shown in Fig. 1. At the reader's right, in this figure, is seen the cat-board resting upon an inclined supporting-frame. Two glass tubes are

shown in front and to the left of the cat-board. The lower tube was inserted in the right ventricle through the pulmonary artery, and carried the outflow from the coronary vessels into a porcelain dish. The blood usually escaped from this tube in drops. Each drop, as it fell, struck on a thin aluminium plate of triangular shape, 17 mm. long and 14 mm. wide at the base, fastened on the lever of a Marey tambour. The plate was bent in such a way that the blood could not remain upon it, but ran off into the dish beneath; the impact of the falling drop caused an air wave in the tambour, which was

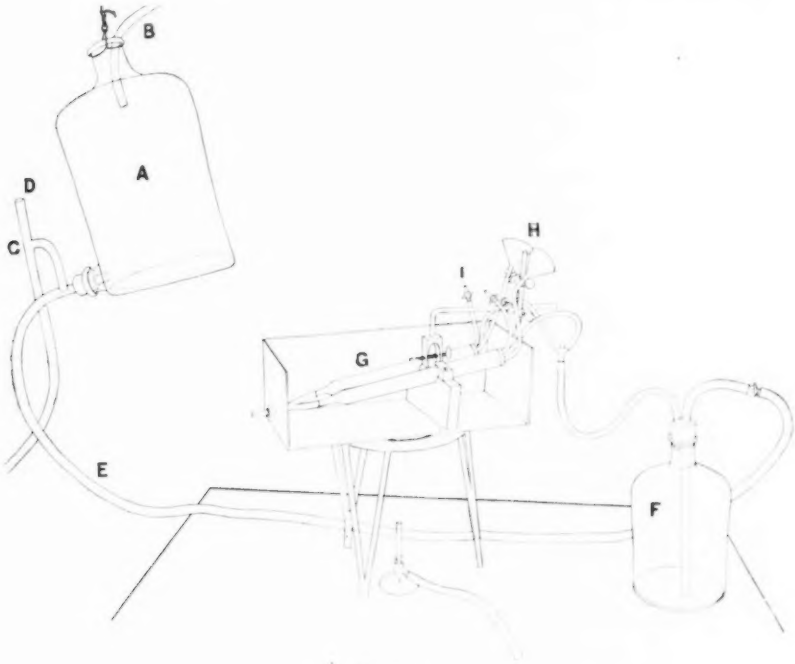

FIGURE 2.

transmitted to a very sensitive recording tambour, the lever of which wrote on the smoked paper of the kymograph. The upper glass tube was inserted in the left ventricle through the left auricular appendix and the mitral valve, and, being filled with normal saline solution, as were the ventricles and the connecting tubes, transmitted the changes of pressure in the ventricle to a Hurthle membrane

manometer, the lever of which wrote on the smoked paper just above the writing of the drop-recorder. A side branch of the manometer led to the atmospheric air and served to place the ventricle at atmospheric pressure. For convenience, this tube has been drawn upright; in actual use, however, the mouth was brought down to the level of the ventricle; the manometer was also at this level. A second side branch led to a Mariotte flask filled with normal saline solution. By raising the flask the pressure in the ventricle could be increased, the amount of pressure being fully recorded by the manometer. The time was recorded by the Jaquet chronometer seen upon the round-base stand in front of the kymograph. The transverse rod, placed at the upper part of the cat-board, supported several hooks which served to lift up the skin at the sides of the thoracic opening, forming thus the walls of a cavity, at the bottom of which lay the heart. This cavity was constantly irrigated with normal saline solution at the body temperature. After filling the cavity, the saline solution escaped into a pan (not shown in the drawing) placed under the supporting-frame.

The apparatus employed for feeding the coronary vessels with blood at the normal temperature and pressure is shown in Fig. 2. The water bottle *A* received a constant stream of tap water through the tube *B*. On reaching the level of the outflow tube *C*, the surplus water passed away into a sink. A constant level was thus maintained in the water bottle. Siphonage was prevented by the upright open tube *D*. A part of the surplus water entered the tube *E*, leading to the pressure bottle *F*, the air in which was subjected to a constant pressure by the column of water in this tube. The compressed air in the pressure bottle was employed to drive the blood from the warming reservoirs through the shortest possible connecting tube into the aorta. The semilunar valves were thereby closed, and, as all the branches of the aorta except the coronary arteries had previously been ligated, the blood was forced through the coronary vessels into the right side of the heart, whence it escaped through a cannula in the pulmonary artery, the venæ cavæ and the vena azygos having been tied. As a rule, the outflow was in drops, and the record of these drops was taken as the measure of the volume of the coronary circulation. Experiments by Magrath and Kennedy,[1] to which the reader is referred, have shown that the size of the drops does not vary enough to make this procedure unsafe.

[1] Magrath and Kennedy: *loc. cit.*, p. 18.

The warming reservoirs, *G*, were filled through the funnel tubes, *H*, the air tubes, *I*, being opened during the filling process. The tubes not furnished with stopcocks were closed with long-handled compression forceps.

The cannula placed in the innominate artery was connected with a mercury manometer, in order that the blood-pressure in the aorta might be observed. The pressure apparatus maintained this pressure with great constancy at any level desired. The manometer served also to assure the observer of the proper closure of the aortic valves. The pressure would fall when these valves were insufficient. At the same time the pressure in the membrane manometer, connected with the interior of the ventricle, would rise. But the aortic valves were seldom insufficient.

The method I have just described is open to the objection that only the blood escaping from the coronary vessels into the right heart was taken as the measure of the volume of the coronary circulation. A slight error is thus introduced, for the coronary blood is discharged not only through the coronary veins but also through the veins of Thebesius, and the veins of Thebesius open into all the chambers of the heart, the left as well as the right. The method employed by me registered the outflow from the right heart, but omitted that from the left. Furthermore, the normal saline solution used to distend the left side of the heart could enter the veins of Thebesius present on this side, pass through the branches that connect them with the coronary veins, and flow with the coronary blood into the right heart, thus making part of the recorded outflow. Yet these errors did not seriously impair the method for the purpose for which it was devised, as the number of the vessels of Thebesius opening into the left heart is too small, and the circulation through them too slight, to be of practical importance in these experiments.[1]

A factor of much greater difficulty was the unexpectedly powerful stimulation of the ventricular muscle by the distention of the ventricle. Hearts that had ceased to contract, although fed through their coronary vessels with defibrinated blood at uniform temperature and pressure, often suddenly awoke to new contractions when the intraventicular pressure was raised; and hearts that were beating feebly

[1] The circulation through the vessels of Thebesius from the left ventricle and auricle, though relatively too small to affect seriously the method here described, may be of importance in the nutrition of hearts in which the coronary arteries are obstructed. See the experiments of F. H. Pratt, this Journal, vol. i, p. 86.

FIG. 3. One half the original size. The flow through the coronary vessels lessens when the left ventricle is distended. The uppermost curve is the pressure in the left ventricle; the middle curve gives the time in seconds; the lowest curve is the volume of the coronary circulation in drops. The first arrow points to the raising of the pressure in the left ventricle; the second, to the lowering of the pressure to that of the atmosphere again.

before distention were often excited to powerful efforts by the stimulus of distention. The increase in the force of beat in consequence of distention seemed at first of no importance, — nothing being then definitely known of the effect of ventricular contraction on the flow of blood through the heart walls. But when, a short time after my experiments were begun, Dr. W. T. Porter[1] proved that the contraction of the ventricle compresses the vessels in its walls, drives out their contents, and forces along the coronary blood, like the strokes of a pump, it was seen that my method could give a pure result only when used with non-beating hearts or with contracting hearts in which distention either failed, for some reason, to stimulate the ventricle to increased action, or overcame the effect of the increased contraction and lessened the volume of the coronary circulation in spite of the favorable influence of the greater force of beat.

The discovery of the force-pump action of the ventricle bore against the method in yet another way. Magrath and Kennedy had shown that lessening the volume of the coronary circulation in the isolated heart lessened the force of contraction, and, to a slight extent, the frequency as well. Hence the distention of the heart, by diminishing the volume of the coronary circulation, may, if the heart is not stimulated to increased contraction by the stimulus of distention, cut down the force and frequency of contraction, and thus, secondarily, still further reduce the coronary flow. But this error is not sufficiently large to be of moment in the present investigation.

The foregoing considerations divide the ques-

[1] PORTER, W. T.: The influence of the heart-beat on the flow of blood through the walls of the heart. This Journal, i, p. 145.

tion in hand into two parts. It is necessary to determine first, whether the distention of the heart checks the flow of blood through the coronary vessels when the organ is at rest. The answer to this question is furnished by Fig. 3, from the experiment of October 24, 1896. The uppermost curve in this figure was drawn by a Hurthle membrane manometer connected with the left ventricle. The rise of three millimetres in this curve indicates an increase of 15 mm. Hg intraventricular pressure; this distention of the ventricle was accomplished by opening a side branch of the ventricular cannula tube, in the manner described on page 218. The middle curve gives the time in seconds. The lowest curve records the number of drops of blood escaping from the coronary vessels into the right heart, — practically, the volume of the coronary circulation. The pressure in the aorta was 78 mm. Hg. When the pressure in the left ventricle is increased, a part of the contents of the coronary vessels is squeezed out, so that the first five or six seconds of the period of distention are marked by an increase in the flow. When the pressure in the ventricle is lowered again, at the second arrow, the sudden dilation of the vessels checks the flow during a few seconds. Apart from these temporary effects, the consequence of

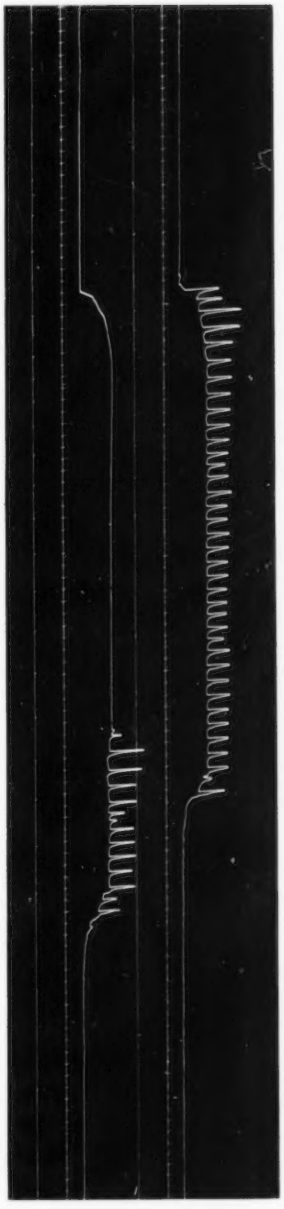

FIG. 4 Two thirds the original size. Two records of the effect of distention of the left ventricle on the flow through the coronary vessels. In each record, the uppermost curve is the pressure in the left ventricle; the middle curve, the time in seconds; and the lowest curve, the number of drops of blood flowing through the heart walls.

distention is a considerable diminution in the volume of the coronary flow.

The number of drops per 20 seconds throughout the experiment was as follows, beginning 60 seconds before distention: —

1–20 seconds,	23	drops.
21–40 "	23	"
41–60 "	23	"

Distention of the left ventricle at 60th sec.

61– 80 seconds,	26	drops.
81–100 "	15	"
101–120 "	14	"
121–140 "	8	"

At the 126th second, atmospheric pressure was restored.

141–160 seconds,	20	drops.
161–180 "	24	"
181–200 "	24	"
201–220 "	23	"
221–240 "	23	"
241–260 "	22	"

The distention of the non-beating heart, therefore, diminishes the flow of blood through the coronary vessels.

I pass now to the effect of distention of the non-beating heart spurred into activity by the stimulus of distention. Fig. 4 illustrates one of these experiments. Two records of the effect of distention on the flow through the coronary vessels are here given. The uppermost curve in each set of three is the pressure in the left ventricle, recorded as described above; the middle curve gives the time in seconds, and the lowest curve the number of drops of blood escaping from the coronary vessels. The stimulating effect of the distention of the heart is very well shown. In the upper intraventricular curve, the pressure rises 27 mm. Hg; in the lower, 35 mm. Hg. In the first experiment (lower tracing), the distention at first fails to call forth contractions; in the second (upper tracing), the heart begins to beat as soon as distended. In spite of the favorable action of the contractions upon the coronary circulation, the effect of the distention, aside from the momentary squeezing out of a part of the contents of the vessels when the pressure in the ventricle is first raised, is to diminish the flow through the coronary vessels.

The second of the two problems before us, namely, whether distention checks the coronary flow in beating hearts, in spite of the favorable influence exerted on the coronary flow by the increased force of

beat called forth by the distention, is already partly answered by the preceding experiment. A more satisfactory reply is afforded by the experiment recorded in Fig. 5. This heart was beating very well before the ventricle was distended. The distention was purposely made very slight, and, as will be seen on examining the curve, is certainly not greater than that observed in hearts distended by natural causes in the intact animal. The base line of the intraventricular pressure-curve rises not more than 5 mm. Hg. The distention increased the force of beat to some extent, the upstrokes becoming taller, but the flow through the coronary arteries fell off nevertheless. The number of drops per twenty seconds was as follows:

1–20	seconds,	22 drops.
21–40	"	22 "

The ventricle was distended at the 40th second of this record.

41–60	seconds,	18 drops.
61–80	"	19 "

The pressure in the ventricle was lowered at the 73d second. From the 61st to the 73d second the rate of flow was 18 drops per 20 seconds.

81–100	seconds,	24 drops.
101–120	"	23 "
121–140	"	23 "

The aortic pressure during the observation was 64 mm. Hg. This curve illustrates also the curious weakening — in some cases amounting to absolute disappearance of the heart-beat — immediately after the distention is withdrawn. The ventricle seems to recover somewhat slowly from this exhaustion.

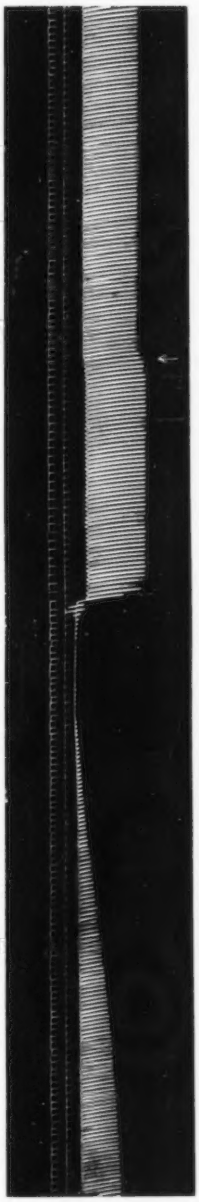

Fig. 5. Two thirds the original size. The uppermost curve is the pressure in the left ventricle; the middle curve, the time in seconds; the lowest curve, the volume of the coronary circulation. The arrow marks the raising of the intraventricular pressure.

It appears, then, that moderate distention may diminish the flow of blood through the walls of even the contracting heart.

These experiments have demonstrated, therefore, that the volume of the coronary circulation is diminished by the distention of the ventricle. This diminution, however, is overcome, in some cases, by the increase consequent on the greater force or frequency with which the ventricle may contract in response to the stimulus of distention.

My material is not sufficient for a discussion of the manner in which the diminished coronary flow is brought about, whether by mechanical influences or by the action of vasomotor mechanisms.

In conclusion, it is with great pleasure that I acknowledge my indebtedness to Dr. H. P. Bowditch, for permission to work in the Laboratory of the Medical School, and to Dr. W. T. Porter, at whose suggestion the investigation was undertaken, and who was constantly ready with kind advice and assistance.

THE CHEMICAL COMPOSITION AND NUTRITIVE VALUE OF SOME EDIBLE AMERICAN FUNGI.

By LAFAYETTE B. MENDEL.

[*From the Sheffield Laboratory of Physiological Chemistry, Yale University.*]

THE collection and spreading of information regarding fungi has lately received considerable attention. The efforts in this direction have been confined for the most part to descriptions of the common species, their peculiarities of growth and distribution. Especial consideration has been devoted to the variations among the fungi as regards toxicity; but accurate statements regarding chemical composition and possible nutritive value are largely wanting. The following extract from a recent monograph will serve to illustrate the current opinions. In referring to the edible mushrooms, it states: "The general opinion is, that mushrooms constitute a very nutritious and sustaining diet. Chemical analysis and personal experience indicate this. The former has shown that in their dry matter they contain from twenty to fifty per cent of protein or nitrogenous material. They may, therefore, well be called a kind of vegetable meat, and be used as a substitute for animal food. Like other vegetables they are largely composed of water, which is from eighty to ninety per cent of the whole. . . . The presence of so much nitrogenous material induces rapid decay and loathsome decomposition in them. . . . A hearty meal on mushrooms alone would be about as reasonable as a dinner on nothing but beefsteak, and might be expected to be followed by similar ill consequences."[1]

In view of the increasing importance of fungi as articles of diet, the writer has gladly followed the suggestion of Professor Chittenden to ascertain something more definite regarding the composition of edible mushrooms, with particular reference to their nutritive qualities.

[1] PECK, C. H.: Report of the New York State Botanist, 1895, p. 113. Cf. also PECK: Mushrooms and their uses, 1897, p. 4. For the source of the statements quoted Professor Peck has referred the writer to the Atlas of Champignons, by Richon and Rosé, and to Les Champignons, by Cordier — both of which it has been impossible to consult.

Methods. — Specimens were obtained from various sources, and in some instances different samples of the same species were examined.[1] The common methods of studying the composition of agricultural products have been adopted, the directions given by the Association of Official Agricultural Chemists being closely followed in most instances.[2] The mushrooms were cut up finely and thoroughly mixed. Samples were taken for the determination of moisture, while the bulk of the material was dried on a water bath and then ground up to a fine powder. Dried to constant weight at 105° C., this served as material for analysis.

Ash was determined in the usual way, the incineration being carried on with the lowest possible heat. The mushrooms employed were previously cleaned with considerable care, and thus an excess of inorganic impurity, such as sand, was avoided.

Ether extract was obtained by treating the material with anhydrous and alcohol-free ether in a Soxhlet extractor for sixteen hours, the extract being finally dried in vacuo to constant weight. Recently Bugdanow[3] has shown that this method is insufficient to remove the last traces of fats completely from some vegetable materials, even when they are finely divided. The error is not sufficiently large, however, to affect the general conclusions from the analyses. In order to examine the extract for cholesterin it was saponified in the usual way with alcoholic potash. Cholesterin (or closely allied substances) was detected by Salkowski's reaction; but the method of separation employed obviously does not exclude the possibility of this substance existing in combination with fatty acids in the fungi.[4]

Crude fibre was determined according to Wiley's method,[5] in the residue left after extractions with ether.

Total nitrogen was found by the Kjeldahl method, duplicate determinations always showing a very close agreement. It is customary in agricultural analysis to express the results thus obtained, and

[1] Acknowledgment is gratefully made of specimens obtained through the courtesy of Mr. Hollis Webster, of Cambridge, and Captain McIlvaine, of Philadelphia. The material used has in every case been identified, or verified, by Dr. A. W. Evans, to whom our thanks are due.

[2] See WILEY, H. W.: Agricultural analysis, 1897.

[3] BUGDANOW: Archiv f. d. ges. Physiol., 1897, lxviii, p. 408.

[4] Cf. HÜRTHLE, K.: Ueber die Fettsäure-Cholesterin-Ester des Blutes. Zeitschr. fur physiol. Chemie, 1896, xxi, p. 352.

[5] WILEY: Agricultural analysis, 1897, iii, p. 304.

multiplied by the factor 6.25, as "crude protein." The latter term is thus made to include albuminoids and extractive bodies as well as the proteids proper.[1] Not only do these individual groups possess quite variable significance as foods, but this investigation has further demonstrated that such calculations may lead to quite erroneous conclusions. In the mushrooms, at least, a considerable part of the nitrogen probably exists as non-proteid nitrogen, a portion even belonging to the so-called crude-fibre, or cellulose elements of the fungi.[2] In a large number of our analyses an attempt has been made to separate the nitrogen of the extractive bodies (amide-nitrogen, etc.) by treating a portion of the material repeatedly with boiling 85 per cent alcohol, so long as anything could be removed. The nitrogen content of the alcoholic extract having been determined, and then calculated on the material used, is designated as *extractive nitrogen*.[3] The amount of *alcohol soluble material* was ascertained at the same time, by filtering the undissolved extraction residues on weighed filters and drying at 105° C. to constant weight. The difference between the total nitrogen and extractive nitrogen is provisionally given as *protein nitrogen*, though, as stated above, there is at present no justification for expressing the results as pure protein. Indeed, as will be pointed out later, this so-called protein nitrogen, in the present instance, contains a large proportion of nitrogen in a form wholly unavailable for the nutrition of the body.

Soluble carbohydrates were determined in an approximate manner by extracting the dry substance repeatedly with hot water and then boiling the extract for ten hours with hydrochloric acid of two per cent resulting strength. The sugar was determined as dextrose in the neutralized fluid, by the Allihn gravimetric method.

Experimental Data. — *Coprinus comatus* (Shaggy coprinus). The specimens were freshly gathered and had not yet turned "inky." They varied very widely in size, thirty-six mushrooms weighing 1485 grams, of which 980 grams belonged to the caps (pileus) and 505 grams to the stems.

[1] Cf. ATWATER, W. O.: Foods. Nutritive value and cost. Farmers' bulletin, No 23, pp. 5, 6. U. S. Dept. of Agriculture, 1894.

[2] WINTERSTEIN: Berichte der deutsch. botan. Gesellsch., xi, p. 441; also Zeitschr. für physiol. Chemie, 1894, xix, p. 521; 1895, xxi, p. 134; GILSON: La cellule, xi, 1er fascicule.

[3] Cf. MÖRNER, C. Th.: Zeitschr. fur physiol. Chemie, 1886, x, 506.

The average weight of a fresh specimen was thus:

Pileus	27 grams.
Stem	14 "
Total weight	41 "

A specimen which had attained the average growth weighed:

Pileus	43 grams.
Stem	25 "
Total weight	68 "

An analysis yielded the following results:

Water	92.19 per cent.
Total solids	7.81 "
The dry substance contained:	
Total nitrogen	5.79 per cent.
Extractive nitrogen	3.87 "
Protein nitrogen	1.92 "
Ether extract	3.3 "
Crude fibre	7.3 "
Ash	12.5 "
Material soluble in 85 per cent alcohol	56.3 "

Coprinus atramentarius (Inky coprinus). Two separate, freshly gathered lots of this species were examined. The one (*a*) contained six young small specimens weighing 5.5 grams, or 0.9 gram each; the other (*b*) contained eight mushrooms weighing 12 grams, or 1.5 grams each. An analysis gave:

	a. Per cent.	*b.* Per cent.
Water	92.31	94.42
Total solids	7.69	5.58
The dry substance contained:		
Total nitrogen	4.68	4.77
Ether extract	3.1	5.7
Crude fibre	9.3	....
Ash	16.8	20.1

Morchella esculenta (Common morel). Two lots of this species were obtained from Stockbridge, Mass. (*a*) The specimens were of full size. Thirteen morels weighed 195 grams, or an average of 15 grams each. (*b*) Small, young morels. An analysis gave:

	a. Per cent.	*b.* Per cent.
Water	89.54	91.24
Total solids	10.46	8.76
The dry substance contained:		
Total nitrogen	4.66	5.36
Extractive nitrogen	1.17	....
Protein nitrogen	3.49	....
Ether extract	4.8	7.5
Crude fibre	8.7	9.5
Ash	10.4	13.6
Material soluble in 85 per cent alcohol	29.3	....

In the same species Pizzi[1] has found 0.575 per cent nitrogen, a figure in close agreement with the above results when calculated upon the fresh material, viz. (*a*) 0.48 per cent N; (*b*) 0.47 per cent N.

Polyporus sulphureus (Sulphury polyporus). The specimens were obtained from Pennsylvania. An analysis gave:

Water	70.80 per cent.
Total solids	29.20 "
The dry substance contained:	
Total nitrogen	3.29 per cent.
Extractive nitrogen	1.06 "
Protein nitrogen	2.23 "
Ether extract	3.2 "
Crude fibre	3.0 "
Ash	7.3 "
Material soluble in 85 per cent alcohol	27.8 ....

Pleurotus ostreatus (Oyster mushroom). This mushroom is obtainable in large quantities, and though somewhat tough in texture, is universally classed with the edible species.

[1] PIZZI: Botanischer Jahresbericht. 1889. p. 316.

Specimens gathered from a tree in New Haven contained:

Water	73.70	per cent.
Total solids	26.30	"
The dry substance contained:		
Total nitrogen	2.40	"
Extractive nitrogen	1.27	"
Protein nitrogen	1.13	"
Ether extract	1.6	"
Crude fibre	7.5	"
Ash	6.1	"
Material soluble in 85 per cent alcohol	31.5	"

Clitocybe multiceps. Peck. The material was collected near Boston, in June, 1897. A portion of small, young specimens was analyzed separately. The results follow:

	Young Specimens.	*Full-grown Specimens.*
	Per cent.	Per cent.
Water	89.61	93.49
Total solids	10.39	6.51
The dry substance of the full-grown specimens contained:		
Total nitrogen	5.36	per cent.
Extractive nitrogen	3.38	"
Protein nitrogen	1.98	"
Ether extract	6.0	"
Crude fibre	9.6	"
Ash	11.5	"
Material soluble in 85 per cent alcohol	57.2	"

A portion of the mushrooms was separated into stems and caps and each analyzed separately, with the following results:

	Stem.	*Pileus.*
	Per cent.	Per cent.
Water	94.07	92.68
Total solids	5.93	7.32
Total nitrogen in dry substance	3.92	5.84
Ash in dry substance	12.98	10.82

The relatively higher content of nitrogen in the pileus corresponds with the distribution of proteid as shown by histochemical examination. In Agaricus campestris, Boletus edulis, and Boletus scaber, C. Th. Mörner has found similar differences between the nitrogen content of caps and stems.[1]

Hypholoma candolleanum.[2] The specimens were obtained from East Milton, Mass., in June, 1897. A few small, young specimens were also obtained from Brookline, Mass. Analyses follow:

	Full-grown Specimens.	*Younger Specimens.*
	Per cent.	Per cent.
Water	88.97	91.97
Total solids	11.03	8.03
The dry substance contained:		
Total nitrogen	4.28	4.44
Extractive nitrogen	1.79	....
Protein nitrogen	2.49	....
Ether extract	2.5	....
Crude fibre	12.1	....
Ash	13.9	19.9
Material soluble in 85 per cent alcohol	44.4	....

Agaricus campestris (Common mushroom). Two varieties of the common mushroom were collected in New Haven. Fifteen specimens of one variety weighed 42 grams, an average weight of 2.8 grams each. The analysis gave:

	a.	*b.*
	Per cent.	Per cent.
Water	87.88	92.20
Total Solids	12.12	7.80
Total nitrogen in dry substance	4.42	4.92
Ash in dry substance	11.66	17.18

[1] Mörner, C. Th.: Zeitschr. fur physiol. Chemie, 1886, x. p. 510.

[2] The specimens corresponded with those described under this name by Stevenson in his work on British Hymenomycetes. Mr. Hollis Webster has informed the writer that Professor Farlow is inclined to regard them as H. apendiculatum.

Regarding the differences in nitrogen content of cap and stem, compare the remarks under Clitocybe multiceps.

Marasmius oreades (Fairy-ring mushroom). Twenty freshly gathered specimens (from New Haven) weighed 9 grams, an average weight of 0.45 grams each. The analysis gave:

Water	74.96 per cent.
Total solids	25.04 "
Total nitrogen of dry substance	5.97 "
Ash of dry substance	7.23 "

Cortinarius collinitus (Smeared cortinarius). Young specimens gathered in New Haven early in November, 1897. The analysis gave:

Water	91.13 per cent.
Total solids	8.87 "
Total nitrogen of dry substance	3.63 "

Digestion Experiments.—In order to procure further data regarding the nutrient value of the mushrooms, artificial digestion experiments were carried out with seven species of the fungi. The procedure was modified after the Stutzer method. About 2.5 grams dry substance were treated in a flask with 100 c.c. of an artificial gastric juice, containing 0.1 gram very active scale pepsin and having an acidity of 0.35 per cent HCl. The flasks were frequently shaken, and after remaining in a thermostat at 38° C. for twelve hours, the undissolved residue was filtered off, washed free from acid, and again treated in the flask for several hours at 38° C. with 100 c.c. amylolytically active fresh chloroform-water extract of dog's pancreas, a little chloroform being added to prevent putrefaction or fermentation. Sodium carbonate (0.25 gram) was then added, followed by 25 c.c. of a proteolytically active thymolized extract of dry pancreas powder (Kühne's method.[1]) At the end of seven hours the residue was again filtered on a weighed filter, washed thoroughly with hot water, and dried at 105° C. to constant weight. *Undigested residue* was thus determined, and the nitrogen content ascertained by the Kjeldahl method and expressed as *nitrogen in residue*. The results expressed in percentages of dry substance are tabulated below.

Discussion of the Analytical Data. *Nitrogen and Protein.* From the results obtained it is evident that the nitrogen (and proteid) content of the mushrooms (or at least those species examined) is considerably smaller than is ordinarily stated. Thus Pavy, quoting from Payen's analyses, announces that in the dried state "mushrooms contain 52 per cent, morels 44 per cent, white truffles 36 per cent, black truffles

[1] See CHITTENDEN and CUMMINS: Studies from the laboratory of physiological chemistry, Yale University, i, p. 109.

Species Digested.	Dissolved substance. Per cent.	Undigested residue. Per cent.	Nitrogen in residue. Per cent.	Total nitrogen. Per cent.	Total nitrogen soluble. Per cent.	Total nitrogen insoluble. Per cent.
Coprinus comatus . . .	73.79	26.21	4.21	5.79	4.69	1.10
" atramentarius .	71.84	28.16	2.79	4.68	3.90	0.78
Clitocybe multiceps . .	62.43	37.57	1.96	5.36	4.63	0.73
Hypholoma candolleanum	68.02	31.98	3.63	4.28	3.12	1.16
Morchella esculenta . .	50.58	49.42	4.16	4.66	2.61	2.05
Pleurotus ostreatus . .	40.57	59.43	1.39	2.40	1.58	0.82
Polyporus sulphureus . .	45.00	55.00	1.05	3.29	2.71	0.58

31 per cent, nitrogenous matter."[1] In a number of species we have determined not only the total nitrogen, but also the extractive (non-proteid) nitrogen as well as the nitrogen in the residue insoluble after artificial gastric and pancreatic digestion. The "protein" nitrogen multiplied — after deduction of the nitrogen in the undigested residue — by the factor 6.25 will give an approximation to the amount of proteid material available through the digestive processes going on in the alimentary canal, and thus throw some light on the true nutritive value of the mushrooms. It is here assumed that the nitrogenous bodies soluble in alcohol are likewise soluble in the digestive fluids; as to the possible presence of alcohol soluble proteids like zein, gliadin, etc., definite information is wanting at present.

The first table following gives a summary of the nitrogen content of various species; in the second table the amount of available proteid has been calculated in the manner referred to.

In considering the relatively high nitrogen content of the residue resisting digestion, it is to be noted that this is not necessarily derived from unattacked proteids. Winterstein[2] and others have shown that the "cellulose" preparations obtained by the usual methods from various fungi contain a considerable percentage of nitrogen; thus a preparation from Boletus edulis contained 5.5 per cent N, and this substance, like similar preparations from Agaricus campestris, Morchella esculenta and other forms, yields glycosamin, $C_6H_{13}O_5.NH_2$, on de-

[1] Pavy: Food and dietetics, 1881, p. 187.

[2] Winterstein: *loc. cit.*; also, Berichte der deutschen chemischen Gesellschaft, 1894, xxvii, p. 3113; xxviii, p. 167.

composition with HCl. It is thus allied to the chitin found in the animal kingdom; further investigation in this direction is highly desirable.

I.

The percentages are calculated on the dry substance.	Total nitrogen. Per cent.	Extractive nitrogen. Per cent.	"Crude protein" nitrogen. Per cent.
Coprinus comatus	5.79	3.87	1.92
Pleurotus ostreatus	2.40	1.27	1.13
Morchella esculenta	4.66	1.17	3.49
Hypholoma candolleanum	4.28	1.79	2.49
Clitocybe multiceps	5.36	3.38	1.98
Polyporus sulphureus	3.29	1.06	2.23
Agaricus campestris — *a*	4.42	....	....
" " *b*	4.92	....	....
Coprinus atramentarius — *a*	4.68	....	....
" " *b*	4.77	....	....
Morchella esculenta (young)	5.36	....	....
Marasmius oreades	5.97	....	....
Cortinarius collinitus	3.63	....	....
Hypholoma candolleanum (young)	4.44	....	....

II.

The percentages are calculated on the dry substance.	Nitrogen insoluble in 85% alcohol. Per cent.	Nitrogen in residue from digestion. Per cent.	Nitrogen of proteid dissolved in digestion. Per cent.	Digestible proteid ($N \times 6.25$).
Morchella esculenta	3.49	2.05	1.44	**9.00**
Hypholoma candolleanum	2.49	1.16	1.33	**8.31**
Coprinus comatus	1.92	1.10	0.82	**5.12**
Clitocybe multiceps	1.98	0.73	1.25	**7.81**
Polyporus sulphureus	2.23	0.58	1.65	**10.31**
Pleurotus ostreatus	1.13	0.82	0.31	**1.94**

It is of interest in this connection to compare the results obtained by C. Th. Mörner[1] in an investigation of thirteen species of fungi common in Sweden. Nitrogenous constituents alone were considered, total N and extractive N, as well as digestible and indigestible N being determined by methods analogous to those used in the present research. Mörner's results, summarized in the following table, show a close agreement, in general, with those already given for different American species.

The results are expressed as percentage of dry substance.	N soluble in pancreatic juice.	N soluble in gastric juice.	Digestible Protein-N.	Indigestible Protein-N.	Protein-N.	Extractive-N	Total N.
Agaricus procerus (cap)	0.28	2.71	2.99	1.27	4.21	2.02	6.23
Agaricus campestris (cap)	0.35	3.29	3.64	1.17	4.89	2.49	7.38
" " (stem)	0.10	2.78	2.88	1.09	4.04	1.98	6.02
Lactarius deliciosus	0.21	1.20	1.41	1.05	2.51	0.60	3.11
" torminosus	0.17	0.79	0.96	1.00	1.94	0.58	2.52
Cantharellus cibarius	0.08	0.71	0.79	1.46	2.29	0.40	2.69
Boletus edulis (cap)	0.16	1.94	2.10	0.65	2.73	1.14	3.87
" " (stem)	0.14	1.62	1.76	0.67	2.35	0.95	3.30
" scaber (cap)	0.18	1.48	1.66	0.85	2.54	0.58	3.12
" " (stem)	0.12	0.87	0.99	0.62	1.71	0.48	2.19
" luteus	0.22	0.48	0.70	1.06	1.77	0.74	2.51
Polyporus ovinus	0.08	0.42	0.50	0.84	1.35	0.45	1.80
Hydnum imbricatum	0.08	0.77	0.85	0.76	1.59	0.96	2.55
" repandum	0.15	1.08	1.23	1.55	2.78	0.74	3.52
Sparassis crispa	0.09	0.37	0.46	0.40	0.97	0.21	1.18
Morchella esculenta	0.22	1.97	2.19	1.90	4.18	0.81	4.99
Lycoperdon Bovista	....	3.13	3.13	2.70	5.79	2.40	8.19

Ether Extract. — The amount of ether extract varied from 1.6 to 7.5 per cent in different species, as shown in the following summary of results.

[1] MÖRNER, C. Th.: Zeitschr. fur physiol. Chemie, 1886, x, p. 503.

ETHER EXTRACT.

Species.	Percentage calculated on dry substance.	Cholesterin.
Morchella esculenta (young)	7.5	Present.
Clitocybe multiceps	6.0	"
Morchella esculenta	4.8	"
Coprinus comatus	3.3	"
Polyporus sulphureus	3.2	"
Coprinus atramentarius	3.1	"
Hypholoma candolleanum	2.5	"
Pleurotus ostreatus	1.6	"

Gérard [1] examined the extract from Lactarius vellereus and L. piperatus, and found oleic and stearic acids present both as glycerides and as free acids. Volatile fatty acids were also obtained, together with cholesterin or a closely related body (ergosterin), and evidences of lecithin. In the present research both fats and free fatty acids were found, and cholesterin reactions were obtained in every instance, the quantitative relations apparently varying considerably in the different species.

Alcohol Extract. — The following summary shows the amount of material soluble in warm 85 per cent alcohol in a number of species.

ALCOHOL EXTRACT.

The percentages are calculated on the dry substance.	Percentage of soluble material.	Percentage of nitrogen dissolved.
Clitocybe multiceps	57.2	3.38
Coprinus comatus	56.3	3.87
Hypholoma candolleanum	44.4	1.79
Pleurotus ostreatus	31.5	1.27
Morchella esculenta	29.3	1.17
Polyporus sulphureus	27.8	1.06

[1] GÉRARD: Journal de pharmacie et de chimie, 1890, 5 Série, xxi, p. 408; *ibid.* 1891, xxiii, p. 7. References to the earlier literature will be found in the first of these papers.

Inorganic constituents. — The amount of ash varied somewhat, as shown in the table below. Among the bases present, K, Na, and sometimes Ca are to be found, the K being quite abundant. Iron was always present. Of acids, phosphoric and sulphuric predominated, chlorine being occasionally found.

ASH.

The percentages are calculated on the dry substance.	Per cent.
Coprinus atramentarius — *a*	16.8
" " — *b* (young)	20.1
" comatus	12.5
Hypholoma candolleanum — *a*	13.9
" " — *b* (young)	19.9
Morchella esculenta — *a*	10.4
" " — *b* (young)	13.6
Agaricus campestris — *a*	11.7
" " — *b*	17.2
Clitocybe multiceps	11.5
" " (stems)	13.0
" " (pileus)	10.8
Polyporus sulphureus	7.3
Marasmius oreades	7.2
Pleurotus ostreatus	6.1

Crude Fibre. — Under this name is included the residue resistant to boiling acids and alkalis, and scarcely to be considered as homogeneous in nature. The results of the analyses are tabulated below.

It has already been pointed out that the cellulose of the fungi contains nitrogen in many instances, and Winterstein [1] has shown that the latter is not due to proteids or nucleins mechanically included;

[1] WINTERSTEIN: Berichte d. deutsch. chem. Gesellsch., xxviii. p. 167: Zeitschr. für physiol. Chemie, 1894, xxix. p. 521.

the nitrogen probably belongs to the "cellulose" itself. All attempts to separate the nitrogenous constituent from the portion which yields sugar on hydrolysis have failed.

CRUDE FIBRE.

The percentages are calculated on the dry substance.	Per cent.
Hypholoma candolleanum	12.1
Clitocybe multiceps	9.6
Coprinus atramentarius	9.3
Morchella esculenta (young)	9.5
" "	8.7
Pleurotus ostreatus	7.5
Coprinus comatus	7.3
Polyporus sulphureus	3.0

Soluble Carbohydrates.—A considerable portion of the solids of the mushrooms is made up of soluble carbohydrates, while starch is ordinarily not found. Trehalose, a carbohydrate of the formula $C_{12}H_{22}O_{11}$, and resembling maltose in some respects, has been isolated from a number of species;[1] and in an extensive series of investigations Bourquelot[2] has described a number of carbohydrates including mannite.

In order to get some idea of the amount of soluble carbohydrates present a number of experiments were carried out in the manner described under the methods of analysis. Since trehalose, for example, cannot be quantitatively converted into dextrose by hydrolysis with acids,[3] the results of analysis must be somewhat low. Nevertheless the data may be of comparative interest as indicating a high content of soluble carbohydrate.

[1] WINTERSTEIN: Zeitschr. für physiol. Chemie, 1894, xix, p. 70. The references to earlier literature are given.

[2] These investigations were published in a series of papers in the Comptes rendus and other scientific journals.

[3] WINTERSTEIN: 1894, *loc. cit.*, xix, p. 77.

DEXTROSE FROM HYDROLYSIS OF WATER-SOLUBLE CARBOHYDRATES.

The percentages are calculated on the dry substance.	Per cent.
Pleurotus ostreatus	18.6
Coprinus comatus	18.0
Morchella esculenta	15.3
Polyporus sulphureus	12.2

To what extent these soluble carbohydrates are available for absorption in their natural form or after digestion it is impossible at present to say. Such qualitative tests as were made showed them to be transformed to reducing sugars rather slowly by the action of saliva. The large undigested residues (26–59 per cent) found in artificial digestions likewise suggest that they are not completely transformed in the alimentary canal. Reference may here be made to the observations of Stone[1] in feeding experiments on animals. He found that the pentosans, which are so widely distributed in vegetable foods, are to a marked degree less digestible than the carbohydrates, with which they have usually been indiscriminately classed in analyses.

After the presentation of the preceding analytical data it will scarcely be necessary to draw any elaborate comparison between the fungi and other well-known vegetable substances considered as foodstuffs. It may be well to emphasize the deficiencies of the methods commonly followed in estimating the proteid content of vegetable foods, and to call attention to the erroneous inferences which are consequently drawn regarding the nutrient value of these products. Thus it is not unusual in the construction of dietetic tables to multiply the weight of nitrogen obtained by 6.25 and to express the result as "crude proteids."[2] But even where the precaution has been taken to remove non-proteid nitrogenous bodies by extraction with alcohol, the application of the "proteid factor" (6.25) to the N. of the residue may be quite misleading; for our results have demonstrated that the amount of unavailable nitrogenous material — largely, if not entirely,

[1] Stone: American chemical journal, 1894, xiv. p. 13.

[2] Cf. Wiley: Agricultural analysis, 1897, iii. p. 543.

non-proteid in nature — is frequently equivalent to over half of the non-extractive nitrogen present (cf. Table II, p. 232). When it is remembered that the various species of mushrooms examined contain from 75 to 90 per cent of water, the amount of proteid in them appears strikingly small even when calculated on the total nitrogen in the fungi.[1] For example, Morchella esculenta, a species of average composition as regards total solids (10.5 per cent) and nitrogenous constituents (0.48 per cent N) could contain as a possible maximum only three per cent of proteid, corresponding in this respect with potatoes, peas, green corn, etc.;[2] the vegetarian would thus be obliged to consume several kilos of the fresh morel to obtain the daily requisite of 100 grams of proteid. The expression "vegetable beefsteak" accordingly seems scarcely appropriate when applied to mushrooms in a strictly chemical sense. Moreover, the comparative poverty of many species in proteids is corroborated by the results of other investigations now in progress in this laboratory, the yield of isolated substance being quite small. The fungi thus form no exception to the ordinary classes of fresh vegetable foods; indeed, they take a decidedly inferior rank in comparison with many.

The carbohydrate content of the fungi is relatively high; but until more is known regarding the nature and digestibility of the carbohydrate constituents of various vegetable foods, it will be useless to draw comparisons. As dietetic accessories the edible fungi may play an important part; but investigation has demonstrated that they cannot be ranked with the essential foods.

[1] Cf. Mörner, C. Th.: Zeitschr. für physiol. Chemie, 1886, x, p. 515.

[2] Cf. Atwater, W. O.: Foods: nutritive value and cost, *loc. cit.*, p. 27.

THE RESTORATION OF COÖRDINATED, VOLITIONAL MOVEMENT AFTER NERVE "CROSSING."

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A CURSORY glance at a tabulated list of the voluminous literature[1] treating of the union of nerves after division will indicate that many eminent experimental investigators have zealously re-studied and discussed this subject ever since the historical experimental results of Cruickshank[2] were announced.

An analysis of this voluminous physiological and surgical literature fully substantiates the present consensus of opinion that if the cut ends of the central and peripheral portions of a recently divided mixed nerve be brought into apposition, complete restoration of function may ultimately occur in the peripheral portion. Further, the results of a number of physiologists (Flourens,[3] Bidder,[4] Schiff,[5] Philippeau and Vulpian,[6] Reichert,[7] Howell and Huber,[8] and others) indicate that if the central end of one divided mixed nerve be sutured to the peripheral end of another mixed nerve, union of the two ends may ultimately occur, and the function of the nerve fibres of the peripheral portion be re-established. That is to say, the nerve fibres composing the peripheral portion regain their function of conductivity as well as the property of irritability. Degenerated muscle fibres

[1] For the full bibliography of this subject to 1892 the reader is referred to the paper of Howell and Huber, Journal of physiology, 1892, xiii, p. 335.

[2] CRUICKSHANK: Philosophical transactions, 1795, xvii. See also FONTANA'S description (Sur le venin de la vipère, etc., Florence, 1781, p. 177) of Cruickshank's experiments.

[3] FLOURENS: Recherches expérimentales sur les propriétés et les fonctions du système nerveux, 1824, p. 272.

[4] BIDDER: Archiv für Anat., Physiol., und wissensch. Medicin, 1842, p. 102; Archiv für Anat. u. Physiol., 1865, p. 246.

[5] SCHIFF: Journal de la physiologie, 1860, iii, p. 217

[6] PHILIPPEAU and VULPIAN: Journal de la physiologie, 1864, vi, p. 421 and 474.

[7] REICHERT, E. T.: American journal of the medical sciences, 1885, January.

[8] HOWELL and HUBER: Journal of physiology, 1892, xiii, p. 335; 1893, xiv, p. 1.

innervated by the peripheral portion of the nerve also regenerate and again contract to the stimulus of a nerve impulse.

If successful union of crossed nerves can be obtained, it is evident that when the motor nerve (N) of a group of muscles (M) has united to the peripheral portion of a motor nerve (n) of a group of muscles (m), and *vice versa*, the group of muscles (m) must receive their nerve impulses from that group of spinal nerve cells from which the nerve impulses to the group of muscles (M) formerly emanated. Similarly, the cells of origin of the nerve (n) will supply the nerve impulses to the muscles (M). Although a very decided change in the destination of the impulses from the spinal cells of origin of the two nerves has been produced by the crossing, the central relations of those spinal cells with each other, with other groups of cells, and with the neuraxons of the cells of the cerebral cortex have not been anatomically altered by simple division and suture of the peripheral nerve trunks.

Modern histological methods reveal, to some degree at least, how intricate and how wide-reaching are the connections which exist between the various central nervous mechanisms. Naturally, therefore, the old question investigated long ago by Flourens again arises as to whether, or no, the intricately connected central nervous mechanisms are in reality capable of adjusting themselves to the new state of affairs, so that the individual regains complete coördinate control of the muscles supplied by the crossed nerves. Further, if the nerve to a group of muscles which are rhythmically contracting and relaxing in response to rhythmical nerve impulses discharged by a certain group of nerve cells in the medulla or in the spinal cord, be divided and crossed with the central portion of another motor nerve, will the nerve cells from which the axis cylinders of the latter arise ultimately become a rhythmically discharging group and entirely assume the function of the rhythmically discharging cells after the regeneration of the united crossed nerves and muscles?

With a view of obtaining a definite answer to the two preceding questions, the various experiments described in this paper were performed.

Previous Work on the Subject. — Although various authors, following in the footsteps of Flourens, have divided and crossed different nerve trunks, motor nerves to supposedly sensory nerves and *vice versa*, and mixed nerves to other mixed nerves, none of these authors has investigated the muscular movements that occur in an animal's leg

with crossed nerves when the motor cortex is electrically or otherwise stimulated. Nor do they appear to have studied the effects upon the rhythmical respiratory and other movements of the vocal cords that may follow the crossing of one recurrens with another motor nerve. Most of the earlier investigators busied themselves with the solution of the problem as to whether, or no, sensory nerves would unite to motor nerves and *vice versa*, with the ultimate re-establishment of the function of the united portions. It is almost needless to point out that the results of such attempts do not come within the scope of this paper. Consequently I confine myself to a brief review of those previous observations that more directly bear upon a part of my own experiments.

According to Flourens'[1] description of his celebrated experiments, the two principal trunks of the brachial plexus in a cock were cut, and the peripheral end of the trunk supplying the upper surface of the wing was sutured to the central end of the other trunk, supplying the lower surface of the wing. At the end of several months the bird had regained perfect use of the extremity of the wing, which no longer dragged, and served for flying (?) as well as before the experiment. When the nerves were exposed, they had completely united in the order in which they had been placed, the inferior end of one nerve being continuous with the superior end of the other, and *vice versa*. In describing the physiological investigation of these united nerves, Flourens writes:[2] "I pinched the nerves above the point of their reunion, — the wing moved at once, and the animal cried; I pinched them below, and the animal felt it as before, and his wing moved again; the same thing took place, when I pinched the enlarged point of reunion. And further, when I pinched the superior nerve above the point of reunion, the muscles of the lower surface of the wing contracted; and, on the contrary, the muscles of the upper surface of the wing contracted when I pinched the inferior nerve, — always above the point of reunion."

[1] Flourens: *loc. cit.*

[2] "Je pinçai ces nerfs *au-dessus* du point de leur réunion, l'aile se mut aussitôt, et l'animal cria; je les pinçai *au-dessous*, l'animal le sentit de même, et son aile se mut encore; pareille chose eut lieu, quand je pinçai le *point grossi* de la réunion. Et de plus, quand je pinçais le nerf supérieur *au-dessus du point de la réunion*, c'étaient les muscles de la face inférieure de l'aile qui se contractaient; et c'était, au contraire, les muscles de la face supérieure de l'aile qui se contractaient quand je pinçais le nerf inferieur, toujours *au-dessus du point de la réunion*."

While this result of Flourens appears to be all that one could desire, the observer neglects to state whether the action of the tensors of the patagium, the nerves of which were probably not divided, was taken into consideration and properly excluded. No mention is made of a microscopical, or even of a very careful anatomical examination of the tissue between the necessarily adjacent crossed trunks. If the central and peripheral ends of the nerves in reality united with each other without the formation of a single nerve fibre between the two adjacent points of suture, the fact is all the more remarkable, for no special precautions seem to have been taken at the primary operation to prevent the latter occurrence, although such a new formation will invariably occur, according to the experience of the writer, unless prevented by some such method as is described below.

In other cocks and in a duck, Flourens sutured the central end of the fifth cervical nerve to the peripheral end of the divided vagus, and, after the expiration of a number of months, divided the other vagus. All the birds died in from one to four days after the latter operation. Information regarding the return of irritability to the united nerves is not given, but evidently the vagal functions had not been re-established *via* the nucleus of the fifth cervical nerve.

The experiments on dogs by Philippeau and Vulpian,[1] in which the central end of the vagus was crossed with the peripheral end of the hypoglossal and *vice versa*, only tend to show that mixed nerves of different origin are capable of union, and throw no light upon the positive restitution of voluntary coördinate control of the groups of muscles supplied by the above mentioned nerves. These observers concluded that although the central end of the vagus would unite to the peripheral end of the hypoglossus, the nerve fibres of the peripheral part of the hypoglossus would not recover their connections with their exciting nerve-centre, and the hypoglossus would be but an instrument at the command of the functional centre of the motor fibres contained in the cervical part of the vagus; a conclusion that seems to be substantiated by the results of the later experiments of Reichert.[2]

After suturing in five dogs the central end of one vagus to the peripheral end of the hypoglossus, Reichert found, after the nerves had

[1] Philippeau and Vulpian: Journal de la physiologie, 1864, p. 421.

[2] Reichert, E. T.: American journal of medical sciences, 1885, January.

united, that certain areas were present in the partially atrophied half of the tongue, which contracted synchronously with inspiration or with expiration, and concluded that the motor fibres of the vagus had actually become united to similar fibres in the trunk of the hypoglossal, and that the hypoglossal fibres conveyed impulses which were peculiar to the vagus apparatus.

Rawa[1] has obtained such remarkably incredible results after crossing nerves of different destination, and also nerves of special function, that one would naturally suspect that his observations and methods must be faulty. For instance, we are told in regard to the cats in which the hypoglossus was sutured to the vagus and *vice versa*, "that of the entire number of cats only six survived. Four of these cats (Nos. 4, 7, 9, 10) had the left central stump of the hypoglossus sutured to the peripheral vagus; two, a similar crossing of the nerves on the right side. In two other cats (12 and 14) the central vagus was sutured to the peripheral hypoglossus on the left side, and in cat No. 16 on the right side." At the expiration of 16–20 months, the right vagus was cut in cats 4 and 7, and both animals promptly died within five days. In cat 12, section of the opposite hypoglossal nerve was followed by loss of power to move the tongue. In cat 16, after the opposite hypoglossal was cut, no movements of the tongue were present, but in a few days the tongue was slowly moved, being contracted to the left, but the animal was killed at the end of six weeks. On page 310, cats 9, 10, and 11 are said to have died very shortly after the primary operation, although cats 9 and 10 were previously included among the six (?) cats that survived the section of one vagus. Likewise, one finds that cat No. 8, previously unincluded in the number of cats surviving the section of the right vagus, was operated on 16–20 months after the primary operation, and two centimetres of the left vagus were excised. Five days later, fearing to lose the animal, it was used for an experiment, for the details of which the reader is referred to the original paper. Rawa's experience leads him to conclude that (1) "after the peripheral portion of a nerve supplying a certain muscle has united to the central end of a nerve that supplies another muscle, the function of the former muscle becomes restored. (2) The direction of the voluntary motor impulses may be altered as one pleases, and the impulses will always accommodate themselves to the peripheral nerve endings." As a result of his experiments in crossing the hypoglossal and vagus, he

[1] RAWA: Archiv für Physiologie, 1885, p. 296.

likewise concludes, "that the central nervous mechanisms can innervate organs that formerly did not connect with them, as soon as those organs become connected to them by nervous conductors." "Nerve centres will, by practice, supply exactly what the peripheral organs with which they became connected require of them."

Howell and Huber[1] crossed the ulnar and the median nerves in dogs and succeeded in getting the crossed nerves to unite without the formation of a cicatrix, common to all the ends. They found, to quote these observers verbatim, "that at the second day after the operation, with both median and ulnar cut on the left side high in the arm, and with the ulnar cut on the right side at the level of the elbow, there was very little evidence of any paralysis or even awkwardness." "Before the end of the first week the animal was running around in perfect freedom, and the closest scrutiny could detect no awkwardness of movement except possibly in running rapidly up stairs he would frequently stumble with his front feet; but whether this was due to the unusual innervation of the muscles, or was caused by the over-zealous activity characteristic of young dogs generally, could not be determined." The close relation between the origin and distribution of the median and ulnar nerves led these observers to remark that "a more interesting suture would probably be one between the musculo-spiral and ulnar in which centres of origin of extensor fibres would be obliged to innervate flexor muscles." They considered there was no histological or physiological obstacle to such a union, but considerable awkwardness of movement in the beginning might attend the functional use of the nerve by the animal. Judging, therefore, from the results of Howell and Huber it would appear that such nerves as the ulnar and the median, which innervate in the dog synergic groups of muscles, are not the ones to choose for crossing when it is desired to investigate the return of voluntary coördinated movements in muscles innervated by crossed nerves. The suture of two nerves supplying antagonistic groups of muscles will yield results that can be more accurately interpreted.

From the preceding brief historical review it is evident that there is room for considerable doubt as to whether the central nervous mechanisms concerned in volition and coördination will in reality adjust their nervous discharges so that a grown animal will regain full control of antagonistically acting groups of muscles after their nerve trunks have been crossed.

[1] Howell and Huber: Journal of physiology, 1892, xiii, p. 335.

Methods.— All the successful experiments were performed upon dogs. In two monkeys which I had hoped would prove more suitable than dogs for this variety of experiment, the ulnar and the median nerves were crossed with the musculo-spiral nerve, but as the experiments were not a success, no further mention need be made of them. Ether anæsthesia was employed for every operation, and all the operations except the last were performed with the strictest aseptic and antiseptic precautions. After the cerebral cortex, etc., had been investigated at the final operation, the animals were killed with an overdose of the anæsthetic.

The nerves were divided with a sharp razor and sutured with fine catgut prepared by the writer's formalin method.[1] Usually two to four fine sutures were employed. After the crossed nerves had been sutured, broad pieces of fascia covering the neighboring pectoral and other muscles were dissected off, and both of the apposed crossed nerves were gently wrapped, for about three-quarters of an inch above and below the point of suture, in separate pieces of this thin tissue, which was then sutured with fine catgut sutures to the fascia of the adjacent muscles. In all the experiments upon the ulnar, median, and musculo-spiral nerves, the common branch from the musculo-cutaneous nerve to the median was entirely excised, its point of origin from the musculo-cutaneous being ligated with a silk ligature. The wound was sutured with No. 2 catgut, dressed with bichloride of mercury gauze, the whole limb wrapped in cotton, bandaged, and, finally, put in plaster of Paris. The plaster not only encased the toes, but also covered the shoulder, and passed around the upper part of the thorax and the lower part of the neck. The fore limb was thus kept perfectly at rest for at least three weeks. The plaster bandage was then removed, to be immediately replaced by a clean one that was allowed to remain on the dog for four to six weeks. If any tendency to ulceration became evident after the removal of the plaster, it was again applied for two to four weeks, or longer, until the vitality of the tissues had sufficiently recovered to resist external sources of irritation. Consequently, by carefully protecting the peripheral parts, the majority of the dogs did not exhibit the ulcerative disturbances that are very liable to occur in the unprotected skin of the wrist, toes, etc., after division of the chief nerve supply of that region.

Previous to the operation, it was found that many of the dogs

[1] CUNNINGHAM: New York medical journal, 1895, April 20.

would give the paw, and some of the remainder were easily taught to do it also; a circumstance that was later of great assistance in judging whether, or no, the recovery of coördinated voluntary control of the muscles concerned in that movement had occurred. Other methods of testing, such as running up a flight of steps, holding a bone after the bandaging of the uninjured foot, etc., were also used. For the electrical investigation, a du Bois induction coil by Reininger, Gebbert, and Schall was employed. The primary circuit of the coil was attached to the mains of the 115-volt illuminating current with a sixteen candle-power lamp in series with the primary of the coil; .5 ampere of current was registered by the ammeter when the hammer was in action. During the electrical examination, insulating rubber was placed under the nerves to prevent the escape of current to neighboring nerves.

After the animals had been killed, very careful dissections of the united nerves were made. In the animals referred to in this paper it was found, unless it is specially mentioned to the contrary, that the crossed nerves had united in the position in which they had been sutured, and that they were not united in a common cicatrix. If the adjacent united nerves were at all firmly adherent, the result was considered questionable, and was thus rejected.

Experiments. — I. *Central portion of right ulnar sutured to the peripheral end of the right median; and the central median to the distal ulnar.*

Dog 1.— Operation January 8, 1895. Plaster bandage removed January 12, and wound found to be healing by first intention. On allowing the dog to run about, it did not appear to limp or seem much inconvenienced by the loss of the functional use of the flexors of the right foot and wrist. Careful comparison with the left foot plainly showed that the right wrist was considerably more extended than the left one, and when the dog was standing with this foot resting on the floor, a considerable part of the palmar surface of the metacarpus touched the floor. If both fore-legs were held up, movements of flexion and of extension of the left paw would occur, but the right paw was held in a state of moderate over-extension, the toes being slightly spread apart. When running up a flight of steps, the dog would often stumble, appearing to strike the edge of a step with the over-extended foot. Owing to the development of a few small ulcers on the plantar balls, the plaster bandage was again applied at

the end of a week, over the whole limb and shoulder, with a few turns around the body. In two weeks this plaster was removed, and on February 26 another careful examination of the animal was made. At this date, the over-extension continued. The right forearm was much smaller than the left from atrophy of the flexor muscles. On putting the left paw into a small boot and giving the dog a bone, the bone frequently slipped from under the right paw by which the dog tried to steady it when he attempted to gnaw it. No movements of the flexor muscles could be detected. After anæsthetizing the animal and exposing the crossed nerves, it was found that the central median had apparently united to the distal ulnar as well as could be desired. The bulbous ends of the crossed central ulnar and distal median had separated about three millimetres, but were connected by a delicate grayish thread-like band that was found to consist of new nerve fibres.

Faradic stimulation of the central median above the point of union produced movements in many of the partially exposed muscles innervated by the ulnar, causing ulnar flexion of the wrist and foot. Stimulation of the distal united ulnar three-quarters of an inch below the point of union also produced ulnar flexion, but not until the strength of the current had been considerably increased. Stimulation of the central ulnar with a rather strong current (10 cm.) produced a faint median flexion of the paw. The distal median had not recovered its faradic electrical irritability, and the electrical irritability of the right distal ulnar was much less than that of the left uninjured ulnar.

After exposing the sigmoid gyrus of both cerebral hemispheres, the areas for extension and for flexion of the paw were stimulated after the paw had been flexed and the arm and forearm made immovable by firm fixation; — extension of the paw readily followed the cerebral stimulus. Only a very slight degree of flexion of the paw could be produced by stimulating the fore limb area in the left hemisphere of this dog, although a stimulus sufficiently strong to produce a severe general fit was finally applied. The central ulnar and the central median nerves were then divided above the points of union and stimulated, the results being the same as before their division. The distal median was then divided and the central ulnar stimulated with a strong current; no flexion of the paw was produced. Stimulation of the central median readily produced ulnar flexion.

Dog 2.—Similar to No. 1, but the dog was kept for seventy-five

days. Over-extension of the paw was still present, and the animal was awkward and stumbled when running up the steps. Cutaneous faradic stimulation of the flexors of the paw showed that, although the faradic irritability of those muscles had been nearly recovered, their irritability was less than that of the flexor muscles of the normal left forearm. Faradic stimulation of the exposed united nerves showed that the nerves were irritable both above and below the points of union, and stimulation of the central ulnar produced well-marked contraction of the muscles innervated by the median. Excitation of the central median produced contraction of muscles supplied by the peripheral ulnar which had been crossed with it. Stimulation of the cortical area for flexion of the paw readily produced that movement.

Dog 3.—The right fore limb of this dog was kept for seven weeks in plaster. At the end of fourteen months, a moderate predominance of the extensors over the flexors of the paw was still evident when the animal was carefully examined. The dog also frequently stumbled when attempting to run rapidly up the steps, and though flexor movements of the right paw were plainly to be seen, the movements did not appear to be quite so actively made in the right leg as in the normal left one. Even after this interval of time, the toes of the right foot were still considerably separated when the animal was resting upon that foot. Electrical excitation of the flexor area of the cortex and of the exposed crossed nerves gave results similar to those met with in dog 2, and needs no further comment. The previously atrophied right flexor muscles had evidently nearly completely regenerated, for the forearms of the dog did not perceptibly differ in size nor did the quantitative faradic electrical irritability of the flexor muscles of the forearms differ much.

The preceding results thus corroborate those of previous workers, in that they clearly prove that one mixed nerve may be crossed with and unite with another mixed nerve. They also clearly demonstrate that the peripheral portion of the crossed united nerve recovers its function of conductivity before it recovers the property of electrical irritability. After the nerves have united and the various groups of muscles have regenerated, nervous impulses emanating from the motor cortex of the brain are still capable of causing the cells of the spinal cord from which the central portions of the crossed nerves arise, to discharge impulses that give rise to contractions of the muscles which the crossed nerves supply. But in the dog, as is well

known, the main functional use of the groups of muscles that are supplied by the ulnar and the median nerves is to produce flexion of the foot, the action of the groups of muscles being synergic and also usually synchronous. Consequently, very little, if any, disturbance of voluntary coördinated flexion of the paw would be likely to follow in the dog when the ulnar and the median nerves have been successfully crossed, a conclusion that is fully exemplified by the result obtained in dog No. 3.

II. *Central end of the right musculo-spiral nerve crossed with the distal portions of the ulnar and the median, and* vice versa.

This operation was performed on nine dogs, but in only four dogs were the experiments successful. In one of these four dogs, No. 2, a large, powerful, restless animal, so much swelling and induration of the tissues developed on the dorsal surface of the wrist from constant attempts to walk upon this surface, that it was impossible to definitely judge whether or not the dog was able to voluntarily contract the extensor muscles of the paw. Evidently the animal was not able to extend the paw intentionally, else it would not have continually flexed the foot at each step and come down upon the dorsal surface of the wrist and foot. Subsequent electrical investigation of the nerves and of the cortical centres showed, however, that the crossed nerves had become at least partially united and regenerated, and that they had recovered their conductivity and electrical irritability.

As the experiments on dogs 1, 3, and 4 yielded essentially uniform results, a description of the results obtained in dog 3 will thus apply to dogs 1 and 4.

Dog 3. — Nerves crossed January 20, 1895, and plaster bandage kept on for two weeks. Wound healed by first intention. Plaster reapplied and kept on for four weeks. Muscles of right forearm markedly atrophied and did not respond to cutaneous faradism. In the course of a week, some contraction of the flexors of the paw, which did not fully relax when elbow was extended. The dog continually held the forearm flexed and the foot was not allowed to touch the ground. When given a bone the animal would attempt to steady it in order to gnaw it by resting the outer side of forearm and flexed foot upon the bone, but was not very successful in keeping it firm.

On October 11th, the dog attempted to use the right leg for walking, but whenever he did so, walked on the back of the foot, on the outer

surface of which was a small ulcer. Ether was administered, the crossed nerves exposed, stimulated, and found to have united. Their electrical irritability had been recovered. Many of the flexor and extensor muscles also responded to direct faradization. After closing the wound and applying an antiseptic dressing to it, and also to the ulcer on the foot, the whole limb was put in plaster with the foot extended. At the end of three weeks the plaster was removed, and the wound and the ulcer were found to be healed. From that time until June 29, 1896, the dog was frequently examined, and the muscles stimulated with mild faradic currents, after previously muzzling the dog, which submitted to this treatment without any especial resistance.

On June 29, 1896, the forearms scarcely differed in size. The muscles of the right forearm seemed to be almost completely regenerated. The right paw was held partially flexed, but when it was carefully observed after steadying the forearm at the elbow, alternating movements of flexion or extension of that paw could be readily seen to occur. When the dog was ordered to give this paw, the animal lifted up the forearm, but instead of extending the foot, the latter was very visibly flexed. Every time the dog walked, the right leg was advanced but the paw was quickly flexed. When a bone was given to the dog, after inserting the left foot in a boot and immobilizing the left wrist by means of a small splint, the movement of the muscles of the right forearm appeared to be so extremely incoördinated that the animal finally held the bone by resting the middle of the forearm upon it. Irregular movements of the adductors and abductors of the toes were also noticed. It should be remarked that this dog had exhibited the above movements early in February, 1896, but certainly no improvement in the coördination of the movements had occurred when the above final examination was made.

The dog also seemed to have recovered sensation on all surfaces of the foot, but the various tests with clips, etc., for determining whether, or not, the animal could correctly localize the position of the peripheral stimulus gave such conflicting results that I am not able to give an opinion in regard to this subject. After anæsthetizing the animal, exposing the motor cortex of both hemispheres, and firmly fixing both elbows so as to prevent any movement at the elbow joint, the cortical area of the right hemisphere for flexion of the wrist was stimulated with a minimal current, and then the same strength of stimulus applied to the area for flexion in the left hemisphere. Result: Extension of the right wrist. Stimulation of the

extensor area, a little further forward in the sigmoid gyrus, produced flexion of the left paw. After repeating this several times the musculo-cutaneous nerve was divided, together with the various flexors and extensors of the forearm; the crossed nerves and blood-vessels being carefully dissected away and protected by cotton wet with warm normal saline solution. After firmly fixing the elbow, the cortex was again stimulated; the flexor area giving rise to contraction of the extensor muscles, the extensor area to flexion of the paw, accompanied apparently by extension of the first phalanges when the current was slightly strengthened.

Two minims of the French oil of absinthe were then injected into the jugular vein. In a few minutes the usual absinthe fit occurred. During the tonic fits the left foot was extended and the right flexed. On immediately excising the small area (extensor) of the left hemisphere, which had been electrically determined to produce flexion of the right foot, the right foot became extended. During another fit the flexor area was excised and the exposed extensor muscles of the right foot no longer participated in the fit.

The preceding results thus conclusively show that the spinal nerve cells from which the musculo-spiral and the ulnar and median motor fibres arise still preserve their connections with the cortical motor mechanisms situated in the sigmoid gyrus.

As far as the cortical areas of this region are concerned, there does not seem to be the least ground for stating that these centres readjust themselves to suit the altered innervation of the groups of muscles which the two united crossed nerves supply. Nor did five months' practice seem to enable the adult dog to regain the functional use of the muscles of the forearm and foot, for, as I have previously remarked, very evident and ample volitional, but incoordinated, movements were visible about five months before the dogs were killed, and none of the dogs showed the least improvement in acquiring any better control of the muscles supplied by the crossed nerves.

III. *Will the rhythmic contractions of certain groups of muscles reappear after union of their motor nerve with the central end of a motor nerve to non-rhythmic muscles?*

To investigate this question, the right recurrens was divided in three dogs as low down in the neck as possible. After carefully freeing the long peripheral portion of the recurrens, it was turned upward around the border of the inferior constrictor of the pharynx and sutured to the

central end of the hypoglossal, which had been cut close to the tongue. The central end of the recurrens was ligated with fine silk, turned toward the root of the neck, and sutured with catgut to the adjacent tissue. Before this operation, these dogs barked very frequently, but after the operation the animals were only able to utter an imperfect, hoarse, stridulous growl. The right half of the tongue was paralyzed, and soon became atrophied and fissured. At the expiration of eight months, it was noticed that the atrophic condition of the right half of the tongue was beginning to lessen, except in dog 3, and also that two of the dogs could move the muscles of that half considerably. At this date, when examined under ether, the regenerated muscles of the right half of the tongue readily responded to faradism. At the end of fourteen to fifteen months, the movements of the tongue seemed to be almost completely restored, except in dog 3, in which the right half of the tongue was permanently paralyzed. Fourteen to fifteen months after the primary operation, the dogs were etherized and the trachea divided just below the larynx. After the insertion of a tube with a short rubber pipe attached to facilitate the administration of the anæsthetic, the anterior composite convolution of both hemispheres was exposed and stimulated with an electrode, the points of which were set one millimetre apart. By carefully adjusting the narcosis, using a stimulus just strong enough to cause the vocal cord to nearly approach the middle line, and carefully removing fluid on the convolutions before applying the electrodes, it was perfectly possible to obtain from both hemispheres adduction of the left vocal cord without any accompanying movements of the tongue when the junction of the præcrucial gyrus and the upper extremity of the anterior composite was stimulated.

When carefully observed[1] from below, or from above, through the widely opened mouth, the right vocal cord was seen to be perfectly immovable. In all of the dogs its position seemed to be about midway between adduction and abduction. When the central hypoglossal or the recurrens which had become grafted to it were stimulated, very evident movements of adduction or of abduction would occur. Sometimes the cord would begin to abduct and then suddenly adduct. When the above-mentioned focus in either hemisphere was stimulated, no movement of the right vocal cord followed unless a very strong

[1] In this connection, the writer wishes to thank Professor Frederic S. Lee for his kindness in carefully observing the movements of the vocal cords on various occasions.

current (secondary at 4 cm.) was applied. With this strong stimulus, movements of the tongue and of swallowing also occurred. Minimal stimulation of the left anterior composite gyrus farther back, where it is joined by the supra-sylvian, was followed by bilateral movements of the split tongue, with adduction, or frequently with abduction, of the right vocal cord. The rhythmical movements of the left were not interrupted. Stimulation of the corresponding area of the right hemisphere produced bilateral movements of the tongue with moderate abduction of the right cord. The left cord did not respond. With the coil at 3 cm., adduction of the right cord occurred.

The left recurrens was then divided, in order to stop the respiratory movements of the left cord, and the above-mentioned regions again stimulated. The movements of the right cord accompanying the movements of the tongue were then more striking, but stimulation of the hemispheres at Krause's laryngeal centre did not produce a movement of the right cord, unless, as previously stated, a current sufficiently strong to produce violent efforts of swallowing was employed.

After killing the dog with the anæsthetic, a dissection of the united nerves disclosed the fact that not only had the sutured recurrens united to the central hypoglossal but that from the latter numerous outgrowths had grown to the base of the tongue and had evidently united with the old peripheral hypoglossal stump. The regeneration of the tongue muscles and the return of voluntary control of the tongue was thus readily explained.

A search for the central end of the right recurrens disclosed the small knobbed end of this nerve about in the position in which it had been sutured; it seemed to be attached to the sterno-thyroid muscle. It had clearly not re-established any connection with the laryngeal muscles.

In dog 3, in which the sutured nerves had been rolled up in a piece of fascia, the outgrowths of nerve fibres from the large hypoglossus had not succeeded in reaching the tongue and producing regeneration of its muscles. When the cortex of this dog was stimulated, no movements appeared in the right half of the split tongue. The right vocal cord responded as in dogs 1 and 2, and no respiratory vocal cord movements could be detected after the section of the left recurrens.

Evidently, therefore, the cells of origin of the hypoglossal nerve do not assume the rhythmical functions of the cells of origin of the recurrens when the latter nerve is made to unite to the central portion of

the former. Clearly, the nerve impulses proceeding from certain nerve centres that innervate the muscles supplied by the recurrens do not shunt off by new or by old paths to the hypoglossal nucleus, when this nucleus, or a part of it at least, is caused to become the nucleus of the recurrens. How much the less likely, therefore, that the hypoglossal nucleus should assume all the functions of the nucleus of the vagus, were that nerve united to the hypoglossus.

To conclude, it is evident that in the dog the central portion of one motor nerve may unite with the peripheral portion of another motor nerve; that the cortical representation of the groups of regenerated muscles supplied by the crossed and united distal nerve is the same as the cortical representation of the groups of muscles that were previously innervated by the central portion before its section; that this cortical representation of the groups, after crossing the nerves, differs from that existing before the nerves are crossed, in that the cortical impulses produce incoördinate movements of the muscles supplied by the united crossed nerve. If two motor nerves supplying two groups of synergic muscles, whose action is to produce almost similar simple movements of an articulation, be crossed, the resultant disturbance of the coördinated mobility of those synergic groups is exceedingly slight, as regards the performance of that particular movement. When groups of muscles innervated by the crossed nerves are of widely different functional use, antagonists, etc., the adult animal (dog) does not regain the power of performing intentional coördinated movements with those muscles, although the fibres of the muscles completely regenerate and recover their former irritability.

Crossing the peripheral portion of the motor nerve of rhythmically contracting muscles to the central portion of the motor nerve of non-rhythmic muscles results in the permanent abolition of the rhythmic action of the former muscles.

In view, therefore, of the foregoing results, it is evident that the central nervous mechanisms do not, as Rawa has claimed, adjust their impulses to suit the altered peripheral innervation, and, by practice, supply exactly what is required of them by the peripheral organs with which they become connected.

PAPAIN-PROTEOLYSIS, WITH SOME OBSERVATIONS ON THE PHYSIOLOGICAL ACTION OF THE PRODUCTS FORMED.[1]

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WHEN papain, the proteolytic enzyme of the papaw plant, was first subjected to careful study by Wurtz and Bouchut,[2] it was compared in its mode of action to trypsin, not alone because it was active in a neutral medium, but especially because of the character of the resultant products. Thus, it was stated that by the vigorous action of papain upon blood-fibrin complete peptonization resulted, with the formation of some leucin in addition. Naturally, at this time (1879) there was no differentiation of proteoses and peptones; hence all that the above statement implied was a conversion of the proteid into a soluble form, precipitable by alcohol and not coagulated by heat nor by acids, although the presence of leucin would certainly suggest the formation of true peptone. Later, Martin[3] pointed out that the enzyme acts vigorously in the presence of sodium carbonate (0.25 per cent) and that as products of digestion there are formed in both neutral and alkaline solutions an intermediate globulin-like body, peptone, leucin, and tyrosin, the last two being formed in small quantity. Here, likewise, the word peptone must be interpreted as meaning simply soluble proteid, and not carrying the distinction which is now known to exist between the proteoses and true peptones. Still later, however, Martin[4] studied the

[1] An abstract of this paper was presented at a meeting of the American Physiological Society held at Washington, May 4, 1897. See Science, N. S., v, June 11, 1897, p. 902.

[2] WURTZ and BOUCHUT: Sur le ferment digestif du Carica papaya. Comptes rendus, 1879, lxxxix, p. 425. WURTZ: Sur la papaine. Contribution à l'histoire des ferments solubles. Ibid., 1880, xc, p. 1379; and xci, p. 787.

[3] MARTIN, S. H. C.: Papain-digestion. Journal of physiology, 1884, v, p. 213.

[4] MARTIN: The nature of papain and its action on vegetable proteid. Journal of physiology, 1885, vi, p. 337.

action of papain on the several proteids occurring with the enzyme in papaw juice, and found that the globulin present there was converted by the enzyme into an albumose (β-phytalbumose), and that this substance was transformed into a peptone-like body, which in turn was converted into leucin and tyrosin. In this case the peptone-like body referred to was presumably a true peptone in the modern acceptance of the term. Working with a somewhat different preparation of papain, the writer[1] observed incidentally that in the digestion of blood-fibrin and coagulated egg-albumin, deuteroalbumose and true peptone predominated among the soluble products formed; *i. e.* peptone, non-precipitable by saturation with ammonium sulphate. More recently, Osswald[2] has also reported that papain as studied by him, gave rise to the formation of peptone in neutral, alkaline, and acid fluids, but that digestion was most complete and rapid in a hydrochloric acid solution. With regard to the latter part of the statement, we are inclined to believe that with most proteids the solvent action of papain is greatest in the presence of sodium carbonate and bicarbonate, although a mixture containing a very little hydrochloric acid may be more active than a neutral solution of the enzyme. Much depends, however, upon the presence or absence of extraneous matters in the ferment-preparation and on the amount of proteid present by which the presence or absence of *free* acid is determined. This question, however, is foreign to our present subject.

If the solvent action of papain on proteids is really due to conversion of the proteids into soluble albumoses and peptone, then its action must be compared with that of a true digestive enzyme and the process itself accepted as a genuine digestive process. In this connection it will be remembered that the corresponding vegetable enzyme bromelin, the proteolytic ferment of pineapple juice, is a true peptone-forming enzyme.[3] In fact, it resembles trypsin very closely in its ability, under suitable conditions, to transform the proteid undergoing digestion into true peptone. It is, of course, hardly to be expected that these vegetable enzymes will prove to be identi-

[1] CHITTENDEN: Papoid-digestion. Trans. Conn. Acad. Arts and Sciences, 1892, ix, p. 321.

[2] OSSWALD: Untersuchungen über das Papain (Reuss). Münchener med. Wochenschr., 1894, No. 34.

[3] CHITTENDEN: On the proteolytic action of bromelin, the ferment of pineapple juice. Journal of physiology, 1894, xv, p. 249.

cal in every respect with the corresponding enzymes of animal origin. Indeed, we already know that in the action of bromelin there are certain minor differences at least in the primary or side-products formed as compared with those resulting from gastric and pancreatic digestion. There has, however, been no reason for doubting the ability of papain to form true peptone, although it must be admitted that since exact methods of separating albumoses or proteoses from true peptones have come into use, no one, so far as we are aware, has isolated the pure peptone or determined the extent or rate of its formation in papain-digestion. On the contrary, within the last few years, the statement has come from several sources that papain has no power whatever to form peptone; that its solvent or digestive action on proteids is limited to the production of proteoses and that peptone is never formed. Thus, Gordon Sharp[1] states, that on warming coagulated egg-albumin with one-tenth its weight of papain and a hundred volumes of water for eighteen hours, no peptone could be detected either by saturating the digestive mixture with ammonium sulphate and testing the filtrate with the biuret test, or by dialyzing the digestive mixture and testing the diffusate (after one hour!) with phosphotungstic acid and by the biuret test. Albumoses, however, were formed. In a second communication[2] the same writer states that by the action of papain upon egg-albumin and serum-albumin in neutral, acid, and alkaline solutions peptone is never formed. Further, the opinion is expressed that the formation of peptone by papain is, on biological grounds, not to be expected, since the function of the ferment in the plant consists merely in transforming proteids into soluble compounds adapted for circulation through the open vessels, whereas in pepsin-digestion, on the other hand, the products of proteolysis must be adapted for absorption by osmosis prior to their distribution and utilization in the body. Lastly, it may be mentioned that Dott[3] in a comparative study of papain and pepsin has likewise found that the former enzyme, unlike pepsin, is not able to form pep-

[1] Sharp: Papain-digestion: Complete absence of peptone. Pharm. J. Transact., liii, p. 633, Edinburgh; Abstract in Chemisches Centralblatt, 1894, i, p. 512.

[2] Sharp: The action of papain upon egg- and serum-albumin in acid and alkaline solution. Pharm. J. Transact., liii, p. 757, Edinburgh; Abstract in Chemisches Centralblatt, 1894, i, p. 830.

[3] Dott: Comparison of the digestive action of papain and pepsin. Pharm. J. Transact., liii, p. 758, Edinburgh; Abstract in Chemisches Centralblatt, 1894, i, p. 831.

tone from egg-albumin. If these statements are correct, then, obviously, papain is quite different in its mode of action from other proteolytic enzymes, and the fact, if such it is, should be clearly established. There would seem to be no great difficulty in arriving at a definite conclusion regarding the matter, and the following experiments have been undertaken with a view to throwing some light upon the question.

In a preliminary experiment, coagulated egg-albumin (from a dozen eggs) was mixed with 800 c.c. of 0.2 per cent sodium carbonate solution, 1 gram of commercial papain added, and the mixture, contained in a closed flask with a little thymol, warmed at 40° C. for three days. Further ferment action was then stopped by boiling, the undissolved matter removed by filtration, the filtrate neutralized with acetic acid, filtered from the precipitate which resulted, and further concentrated. From this concentrated fluid the proteoses were precipitated collectively and completely by saturating the fluid while boiling hot with ammonium sulphate, — carrying out the saturation in a neutral, acid, and ammoniacal fluid successively, as recommended by Kühne[1] for the complete separation of proteoses from peptone. On testing this proteose-free filtrate with the biuret test, giving due heed to the necessity of adding sufficient potassium hydroxide to decompose all of the ammonium salt present, an intense reaction for peptone was obtained. Indeed, it was quite evident from the character of the reaction, that a fairly large percentage of true peptone had been formed.

A similar experiment was tried with coagulated blood-fibrin, this form of proteid being warmed at 40° C. for two days with 1 gram of papain in 800 c.c. of 0.4 per cent sodium carbonate, a little thymol being present. On removal of the proteoses with ammonium sulphate, as described above, a strong biuret reaction was obtained in the filtrate, thus showing the formation of true peptone.

Obviously, one possible danger in experiments of this order, where an alkaline fluid containing so much admixed proteid is warmed at 40° C. for two or three days, is bacterial contamination by which putrefaction may be incited. In the two preceding experiments, thymol was made use of to obviate this danger, but in the next experiment chloroform and sodium fluoride were likewise employed, as follows: —

[1] Kühne: Erfahrungen uber Albumosen und Peptone, Zeitschrift für Biologie, 1892, xxix, p. 1.

1	2	3	4
60 grams fibrin [1]	60 grams fibrin	60 grams fibrin	60 grams fibrin
500 c.c. 0.25% Na_2CO_3	500 c.c. 0.25% Na_2CO_3	500 c.c. 0.25% Na_2CO_3	500 c.c. 0.25% Na_2CO_3
5 c.c. chloroform	2.5 grams thymol	5.0 grams NaF	5.0 grams NaF
1 gram papain	1 gram papain	1 gram papain	1 gram papain, boiled 5 min.

These mixtures were placed in suitably stoppered flasks, shaken thoroughly to insure complete solution of the sodium fluoride, etc., and warmed at 40° C. for twenty hours, with frequent agitation. At the end of the period the mixtures were boiled and filtered, the filtrates neutralized, concentrated, and the proteoses separated as already described by saturation with ammonium sulphate. On testing the filtrates with the biuret test, Nos. 1, 2, and 3 gave a strong reaction for peptone, the reaction in No. 3 being apparently a little the strongest. No. 4, in which the papain was boiled prior to mixing it with the fibrin, gave a purely negative result, thus showing that the peptone reaction in the preceding mixtures could not have come from any admixture contained in the papain itself, nor in the proteid made use of, and that consequently the peptone found must have been formed in some manner during the experiment. Further, this same negative result affords evidence that the peptone detected was not formed by putrefaction; hence it must come from the proteolytic action of the enzyme, which is plainly not hindered by the presence of either chloroform, sodium fluoride, or thymol. Lastly, it should be mentioned that the striking brilliancy of the peptone reactions obtained in Nos. 1–3 precludes the possibility of any other conclusion than that a fairly large proportion of true peptone was formed.

A similar series of experiments was carried out with coagulated egg-albumin, 75 grams of the moist coagulum being used in each mixture, with results wholly in accord with those just described. Further, another series in which fresh, thoroughly washed rabbit's muscle (60 grams in each mixture) was digested gave similar results, the only difference being that in Nos. 1–3 the peptone reaction was even stronger than with the coagulated proteids, as might perhaps be expected owing to the easier digestibility of the former. It is thus quite apparent that papain is a true peptone-forming enzyme, and

[1] Coagulated blood-fibrin.

furthermore is able to exert this action upon various kinds of proteid matter.

What now is the extent to which this formation of peptone may be carried by papain? In the digestion of proteids with pepsin-hydrochloric acid or gastric juice it has been clearly shown that the formation of peptone rarely exceeds 50 per cent; proteoses usually predominate.[1] With alkaline trypsin solution or pancreatic juice, on the other hand, the formation of peptone is much greater, although the hemipeptone formed is eventually broken down by the continued action of the enzyme into amido-acids, etc., leaving only the antipeptone. If papain is a true peptone-forming enzyme, related more closely to trypsin than to pepsin, it follows that under favorable circumstances it might be expected to produce even more than 50 per cent of peptone. It is not to be understood by this statement that papain can be compared with trypsin in rapidity of action; but merely that of the proteid dissolved by papain, under suitable conditions, full 50 per cent might not unreasonably be looked upon as convertible into true peptone by the continued action of the enzyme. The correctness of this view has been tested by several series of quantitative experiments in which the proportion of proteoses and peptones formed has been determined as accurately as existing methods will allow.

The first experiment of this nature may be described as follows: Coagulated egg-albumin, formed by pouring the whites of eggs into boiling water acidified with acetic acid, was washed thoroughly with water, pressed, and finely divided. The content of dry albumin was then determined in a sampled portion by drying at 110° C., and igniting the residue to obtain the amount of ash. By this method 10 grams of the moist coagulum were found to contain 1.9257 grams of dry proteid. Three digestive mixtures were then prepared, each containing 150 c.c. of 0.25 per cent sodium carbonate saturated with chloroform, 50 grams of the moist coagulated albumin and 0.75 gram of active papain. To obviate any error that might be introduced through the presence of albumose, etc., in the papain, a fourth mixture was prepared similar to the above, except that it contained no albumin. All four mixtures were placed in closely stoppered flasks and transferred to a warm chamber, where they were kept at 38–40° C. for vary-

[1] Chittenden and Amerman: A comparison of artificial and natural gastric digestion, together with a study of the diffusibility of proteoses and peptone. Journal of physiology, 1893, xiv, p. 483.

ing lengths of time with occasional agitation. One was allowed to digest for 25 hours, the second was interrupted at the end of 51 hours, while the third mixture and likewise the control were continued for 75 hours. Digestion was stopped by heating the mixture to boiling. It will be noticed in these experiments that the proportion of papain employed was quite small, considering the low digestive power of the enzyme.

The mixtures were analyzed as follows: The undissolved residue, made up largely of an insoluble antialbumid-like substance, together with some unaltered proteid, was collected on a weighed filter, washed thoroughly with water and lastly with alcohol, then dried at 110° C. until of constant weight. The filtrate and washings were then neutralized with dilute acid, and the neutralization precipitate so obtained was collected on a weighed filter, washed with water until free from salts, dried, and weighed. To determine the albumoses, the neutral filtrate and washings were concentrated to a small volume and then precipitated while still hot by saturation with pure neutral ammonium sulphate, giving heed to Kühne's latest modifications of the method.[1] The precipitate was filtered by the aid of a hot-water funnel and washed free from peptone with a hot saturated solution of ammonium sulphate.[2] The precipitate, together with the adherent ammonium sulphate, was then washed into a weighed capsule with hot water, the mixture evaporated to dryness, and finally dried in an air-bath at 110° C. until of constant weight. Obviously, the weight so obtained was the combined weight of the albumoses and ammonium sulphate. To ascertain the value of the latter, the mixture was treated with water containing a little hydrochloric acid, the fluid made up to a definite volume, and in an aliquot portion of the latter the sulphuric acid was determined in the usual manner by precipitation with barium chloride. From the weight of barium sulphate thus obtained the amount of ammonium sulphate was calculated and deducted from the combined weight of the albumoses and ammonium salt. The amount of true peptone formed was obtained in this experiment by deducting the combined weight of the antialbumid and undigested residue, neutralization precipitate, and albumoses from the weight of coagulated proteid used, making the necessary corrections for proteoses, etc., in the papain.

The results from this experiment were as follows, expressed in grams: —

[1] *Loc. cit.* [2] Continued until the filtrate failed to show any biuret reaction.

Period of digestion at 40° C. . . .	25 hours.	51 hours.	75 hours.
Undissolved residue	3.4555	3.1626	. . . .[1]
Neutralization precipitate	0.1692	0.0920	0.0075
Albumoses	2.6167	2.3700	1.2767
	6.2414	5.6246	
Dry proteid used	9.6275	9.6275	9.6275
Peptone formed	3.3861	4.0029	

Expressed in percentages calculated on the dry proteid used, these figures yield the following results: —

Period of digestion at 40° C. . . .	25 hours.	51 hours.	75 hours.
Undissolved residue	35.8	32.8	. . . .
Neutralization precipitate	1.7	0.9	0.1
Albumoses	27.1	24.6	13.3
Peptone	35.4	41.7	
	100.0	100.0	

In considering these figures, emphasis is to be laid upon the fact that the large percentage of undissolved residue noted above is by no means composed mainly of unaltered proteid, but is made up to a considerable extent of a peculiar alteration product which seemingly resembles antialbumid, the formation of which must involve a certain amount of energy on the part of the enzyme. Further, it is to be noted that at the end of twenty-five hours digestion, 62.5 per cent of the proteid is converted into albumoses and peptone, while of these soluble products 56.6 per cent is composed of true peptone, the remaining 43.4 per cent being made up mainly of deuteroalbumose. Moreover, it is seen that as the digestion is continued the proportion of albumoses decreases, peptones being correspondingly increased. To be sure, the figures representing the proportions of peptone formed are obtained by difference, but we see no reason why the methods pursued are not capable of yielding results substantially

[1] Lost by an accident.

correct. Moreover, on testing the three ammonium sulphate-saturated filtrates containing the peptone with the biuret test, the intensity of the reactions obtained corresponded exactly with the above data. In this connection it should be mentioned that even under most favorable conditions the formation of amido-acids or other crystalline decomposition products by papain is very slight.

A second series of experiments similar to the above next demand attention because they help make clear possibly why some observers have failed to find evidence of the formation of peptone by papain. Four distinct mixtures were prepared, each containing 150 c.c. of 0.25 per cent sodium carbonate saturated with chloroform, 50 grams of moist coagulated egg-albumin, and 0.5 gram of papain. The fourth mixture, however, differed from the other three in that the papain was boiled with a portion of the fluid prior to mixing it with the albumin. It thus served as a control to check any possible errors that might arise from the action of the alkali alone on the proteid, or from soluble matter contained in the papain. Of greater importance, however, is the fact that the proportion of papain employed in this series of experiments was considerably less than in the previous series; *i. e.*, 0.5 gram instead of 0.75 gram for every 50 grams of proteid. Furthermore, the papain was a different sample, obtained from a different source, and had been tested solely as to its ability to *dissolve* proteid matter.

The several mixtures were kept at 38–40 C. for varying periods of time, one being removed at the end of 25 hours, the second at the end of 48 hours, while the third and fourth were continued for 72 hours. The mixtures were then analyzed as in the preceding case, with the following results expressed in grams: —

Period of digestion at 40° C. . . .	25 hours.	48 hours.	72 hours.
Undissolved residue	3.8354	3.9759	3.7377
Neutralization precipitate	0.0405	0.0387	0.0100
Albumoses	2.8180	2.3278	2.5176
	6.6939	6.3424	6.2853
Dry proteid used	6.8564	6.8564	6.8564
Peptone formed	0.1625	0.5140	0.5711

In the control mixture, in which the papain had been boiled before mixing it with the albumin, 72 hours at 40° C. resulted simply in the formation of 0.03 gram of neutralization precipitate and a trace only of albumose. The undissolved residue when dried weighed 6.9301 grams, the plus weight being due to the insoluble matter of the papain. The slight corrections made necessary by these data have been embodied in the above figures.

The percentage results calculated on the dry proteid used are as follows: —

Period of digestion at 40° C. . . .	25 hours.	48 hours.	72 hours.
Undissolved residue	55.9	57.9	54.8
Neutralization precipitation . . .	0.6	0.5	0.1
Albumoses	41.1	33.9	36.7
Peptone	2.4	7.7	8.4
	100.0	100.0	100.0

Here, for some reason, the formation of peptone was comparatively slight. Although the amount of papain employed in each mixture was less than the quantity used in the first series of experiments, the ratio of papain to dry proteid was much the same in the two cases. Evidently, the papain made use of in this last experiment was far less active than the preceding preparation, as shown also by the large percentage of undissolved residue. To be sure, considerable albumose was formed, but the enzyme was so lacking in vigor that extensive proteolysis was impossible, and as a result the formation of peptone progressed very slowly. Still, even under these adverse conditions, some peptone was formed — easily recognizable by the biuret reaction — and the proportion increased slowly with continued digestion. There is therefore even in this experiment no confirmation of the statement that papain is unable to form peptone, but merely a suggestion of the necessity of obtaining an active preparation of the enzyme in order to arrive at a true understanding of its proteolytic power.

In a third series of experiments still another preparation of papain was employed: one which preliminary experimentation showed to be quite active. Each mixture contained 150 c.c. of 0.25 per cent sodium carbonate saturated with chloroform, 50 grams of moist

coagulated egg-albumin, and 0.75 gram of papain. A control mixture of albumin, etc., in which the papain was boiled to destroy its activity, was also included in the series. In this series, however, digestion at 40° C. was continued for longer periods, and it is likewise to be noted that the ratio of dry proteid to the papain employed varied from that in the previous experiments. Further, the method of determining the albumoses and peptone was somewhat different from that previously used. Thus, after separating the undissolved residue and neutralization precipitate, the neutral fluid was concentrated and the albumoses precipitated by saturation of the fluid in the cold with pure zinc sulphate after the method of Bomer.[1] This precipitate was collected on a filter, washed thoroughly with a saturated solution of zinc sulphate, after which it was dissolved in water, the solution made up to a given volume, and the nitrogen determined in a fraction of the fluid by the Kjeldahl method. On multiplying the values so obtained by the factor 6.25 the amounts of albumoses present were calculated. The figures for peptone were obtained by difference, but were verified by determination of the nitrogen in the zinc sulphate-saturated filtrates. This, however, was not easily accomplished owing to the presence of so much zinc sulphate; but on diluting the fluid with water we were able to determine the nitrogen in a small volume of the mixture, using the Kjeldahl method, and on multiplying the nitrogen found by the factor 6.25 we obtained values not greatly at variance with those given by difference. It is needless to say that the control mixture was treated in a similar manner and corrections made for nitrogen introduced with the papain, etc. Following are the results obtained expressed in grams: —

Period of digestion at 40° C.	48 hours.	98 hours.	144 hours.
Undissolved residue . . .	0.9487	1.0569	1.1136
Neutralization precipitate .	0.0542	0.0127	0.0152
Albumoses	0.6250	0.6665	0.7506
	1.6279	1.7361	1.8794
Dry proteid used	4.4042	4.4042	4.4042
Peptone formed	2.7763	2.6681	2.5248

[1] BÖMER: Zinksulfat, ein Fällungsmittel für Albumosen, Zeitschr. f. analyt. Chem., 1895, p. 562.

Expressed in percentages, calculated on the dry proteid used, these figures lead to the following results: —

Period of digestion at 40° C.	48 hours.	98 hours.	144 hours.
Undissolved residue . . .	21.5	24.0	25.2
Neutralization precipitate . .	1.2	0.3	0.3
Albumoses	14.2	15.1	17.0
Peptone	63.1	60.6	57.5
	100.0	100.0	100.0

Here we have plain evidence again of the ability of papain under suitable conditions to form relatively large quantities of peptone, the latter, in this experiment, being greatly in excess over all the other products combined. It is furthermore evident that in order to bring out the full proteolytic power of the enzyme (assuming an active preparation) it is necessary that the latter be present in fairly large proportion, *i. e.*, as compared with the proteid matter. When this is the case, as in the present experiment, the element of time is of less moment. In other words, when the ratio of enzyme to proteid is suitable, the maximum digestive action under those conditions is reached in 24–48 hours, and longer exposure at 40° C. fails to increase the proportion of peptone formed. In illustration of this point compare the results of the first and third experiments. In conclusion we think it clearly established that papain is not only a peptone-forming enzyme, but that under proper conditions it is able to transform a large proportion of the proteid matter into true peptone. In confirmation of this statement we have been able to prepare and isolate the pure peptone in quantity sufficient to study some of its physiological properties.

Some Observations on the Physiological Action of the Deuteroalbumose and Peptone formed by Papain.

It has been generally believed for some time past that the primary products which result from the proteolytic action of vegetable enzymes, as well as those formed by the action of superheated water, are somewhat different in nature from the corresponding products

formed by pepsin-acid and by trypsin. Thus, Neumeister[1] has shown that if atmid albumin or atmid albumose, *i. e.*, the albumose formed by the action of superheated water on blood-fibrin, is injected directly into the blood of a dog it appears in the urine wholly unaltered. An ordinary albumose, however, *i. e.*, such as is formed by pepsin or trypsin, when introduced into the circulation (of a dog) appears in the urine more or less hydrated.[2] Thus, proto-albumose appears in the urine in part as deuteroalbumose, while if deuteroalbumose is injected into the blood it appears in the urine as peptone. Peptone, on the other hand, is eliminated wholly unchanged. Neumeister[3] also makes the statement that the products which result from the action of papayotin upon albuminous substances are identical with those formed by the action of superheated water. This implies that the so-called atmid products and the papayotin products are alike in their resistance to the action of pepsin,[4] for it is assumed at least that it is the presence of this enzyme in the kidney which leads to the hydration of the ordinary albumoses during their elimination from the body. In the case of rabbits, where pepsin is wanting in the kidney, the injection of albumoses into the blood is followed by their elimination unchanged (Neumeister).

Moreover, there are certain peculiarities in the chemical composition of the atmid bodies,[5] shared to some degree by the proteoses formed by the action of bromelin[6] — the proteolytic enzyme of pine-apple juice, — which lends favor to the view that these bodies are not quite identical with the proteoses, etc., formed by animal en-

[1] NEUMEISTER: Ueber die nächste Einwirkung gespannter Wasserdämpfe auf Proteine und über eine Gruppe eigenthümlicher Eiweisskörper und Albumosen. Zeitschr. f. Biol., 1890, xxvi. p. 77.

[2] NEUMEISTER: Ueber die Einführung der Albumosen und Peptone in den Organismus. *Ibid.*, 1888, xxiv. p. 272.

[3] NEUMEISTER: *Ibid.*, xxvi. p. 82.

[4] Since this paper was written, there has appeared an article by E. Salkowski, "Ueber die Einwirkung des überhitzten Wassers auf Eiweiss," Zeitschr. f. Biol., 1897, xxxiv. p. 190 (Jubelband zu Ehren von W. Kühne), in which it is stated that the atmidalbumose formed by him from blood-fibrin was not resistant to the action of either pepsin, trypsin, or bacteria, thus differing widely from Neumeister's product.

[5] CHITTENDEN AND MEARA: A study of the primary products resulting from the action of superheated water on coagulated egg-albumin. Journal of physiology, 1894, xv, p. 501.

[6] CHITTENDEN: The proteolytic action of bromelin, the ferment of pineapple juice. *Ibid.*, 1894, xv, p. 249.

zymes. Consequently, it seemed desirable to study with some care the physiological behavior of the albumoses and peptone resulting from papain-digestion with a view to ascertaining what differences of a physiological nature, if any, exist between the latter products and those resulting from animal enzymes.

As has already been pointed out, the soluble products which are formed in the digestion of coagulated egg-albumin with papain are mainly deuteroalbumose and peptone. These were prepared in considerable quantity by digesting the coagulated albumin from four dozen hen's eggs with 9 grams of papain in 2 litres of 0.25 per cent sodium carbonate for 48 hours at 40° C. in the presence of chloroform. The resultant fluid freed from insoluble matter and neutralization precipitate was concentrated to a small volume and the albumoses precipitated by saturation with ammonium sulphate, boiling hot, from a neutral, acid, and alkaline reacting fluid. The precipitate so obtained was dissolved in water, the fluid carefully neutralized, and then dialyzed in running water until wholly free from ammonium sulphate and other salts. The solution was then filtered from a little insoluble matter (heteroalbumose, dysalbumose) concentrated to a small volume, and a portion tested for protoalbumose by saturation of the neutral fluid with rock salt. No precipitate whatever was obtained, consequently the entire volume of fluid was brought to a syrup and the deuteroalbumose precipitated with strong alcohol. After thorough washing with alcohol and ether, the substance was dried at 100° C. making about 20 grams of pure deuteroalbumose.

To obtain the peptone, the ammonium sulphate-saturated filtrate from the albumoses was treated with 50 per cent alcohol, thereby precipitating a large portion of the ammonium salt, while the residual sulphate was removed from the filtrate, after freeing from alcohol, by treatment with barium hydroxide followed by barium carbonate. On evaporating the final filtrate to a syrup and treating with alcohol, the peptone was precipitated more or less gummy, after which it was dehydrated by successive treatments with absolute alcohol and ether, and finally dried at 100° C. About 10 grams of pure peptone were obtained.

Mode of Experimentation. — Our study of the physiological action of the deuteroalbumose and peptone formed above was limited to ascertaining their effects on blood-coagulation, their influence on blood-pressure, and their elimination by the kidneys. In all of the experiments dogs were employed, the animals always being anæsthetized.

Most generally this was accomplished by means of a mixture of equal parts of chloroform and ether, although in some of the experiments morphine sulphate was injected hypodermically followed by the administration of chloroform and ether. In the few cases where morphine was used, it was employed in the proportion of 1 centigram of morphine sulphate for each kilo of body-weight.

The albumose or peptone was introduced either into the left femoral vein or into the facial vein through a cannula connected with a burette. The substance, in the proportion of 0.5 gram per kilo of body-weight, was dissolved in 0.7 per cent sodium chloride solution, the volume of the fluid injected ranging from 30 c.c. to 50 c.c. and never exceeding the latter. The fluid was warmed to 40° C.

To observe the rate at which the blood coagulated, portions about 5 c.c. each were withdrawn at stated intervals from the right femoral artery through a cannula inserted in that vessel, the blood being collected in slender test-tubes to observe the time of coagulation. Each time the blood was withdrawn from the artery the first portion passing out was discarded. Moreover, the cannula was removed and cleaned after each withdrawal of blood. Blood-pressure was registered at the carotid artery, or in some instances at the left femoral artery, using a Hürthle spring manometer and a Baltzar kymographion driven at a slow rate.

Influence on Coagulation of the Blood. — The effects of deutero-albumose and peptone on the coagulation of the blood were observed in eight experiments on dogs ranging in weight from 5 to 11.5 kilos. In the first experiment the dosage of albumose was 0.33 gram per kilo of body-weight, but in four other experiments the dosage was increased to 0.5 gram per kilo, which proportion was likewise used in the three experiments with peptone. Following are the results obtained: —

FIRST EXPERIMENT.

Dog, 9 kilos. 3 grams *deuteroalbumose* in 37 c.c. 0.7 per cent NaCl.
Injection lasted 2 min. 45 sec.
The normal blood coagulated in 3 minutes.[1]

Blood withdrawn	3	minutes	after	injection	of	albumose	coagulated in 30 min.
"	"	9	"	"	"	"	25 "
"	"	22	"	"	"	"	27 "
"	"	28	"	"	"	"	1–2 hours.

[1] The figure given for the coagulation-time of the normal blood is the average of 2–3 determinations.

SECOND EXPERIMENT.

Dog, 6.5 kilos. 3.25 grams *deuteroalbumose* in 50 c.c. 0.7 per cent NaCl.
Injection lasted 3 minutes.
The normal blood coagulated in 10 minutes.

Blood withdrawn	1	minute	after	injection	of	albumose	coagulated	in	1 hr.	27 min.
"	"	4	"	"	"	"	1	"	23	"
"	"	8	"	"	"	"	1	"	19	"
"	"	12	"	"	"	"	1	"	15	"
"	"	18	"	"	"	"	1	"	10	"
"	"	29	"	"	"	"	0	"	58	"
"	"	47	"	"	"	"	0	"	40	"
"	"	49	"	"	"	"	0	"	38	"

THIRD EXPERIMENT.

Bitch, 7.2 kilos. 3.5 grams *deuteroalbumose* in 50 c.c. 0.7 per cent NaCl.
Injection lasted 1 minute.
The normal blood coagulated in 9 minutes.

Blood withdrawn	2	min.	after	injection	of	albumose	was	uncoagulated	at	the end of 18 hrs.
"	"	8	"	"	"	"	"	"	"	"
"	"	25	"	"	"	"	"	"	"	"
"	"	46	"	"	"	"	"	"	"	"

FOURTH EXPERIMENT.

Dog, 7 kilos. 3.5 grams *deuteroalbumose* in 50 c.c. 0.7 per cent NaCl.
Injection lasted 1 min. 15 sec.
The normal blood coagulated in 3.5 minutes.

Blood withdrawn	6	min.	after	injection	of	albumose	was	uncoagulated	at	the end of 36 hrs.
"	"	12	"	"	"	"	"	"	"	"
"	"	18	"	"	"	"	"	"	"	"
"	"	24	"	"	"	"	"	"	"	"
"	"	34	"	"	"	"	coagulated	within	5½	hrs.
"	"	42	"	"	"	"	"	"	3	"

FIFTH EXPERIMENT.

Dog, 11.6 kilos. 5.6 grams *deuteroalbumose* in 40 c.c. 0.7 per cent NaCl.
Injection lasted 45 seconds.
The normal blood coagulated in 9 minutes.

Blood withdrawn	2	minutes	after	injection	of	albumose	coagulated	in	7½	hours.
"	"	4	"	"	"	"	"	"		
"	"	7	"	"	"	"	"	"		
"	"	12	"	"	"	"	"	2 hrs.	36	min.
"	"	17	"	"	"	"	"	2	" 31	"
"	"	26	"	"	"	"	"	0	" 42	"
"	"	36	"	"	"	"	"	0	" 20	"
"	"	45	"	"	"	"	"	0	" 23	"
"	"	55	"	"	"	"	"	0	" 13	"
"	"	65	"	"	"	"	"	0	" 6	"
"	"	75	"	"	"	"	"	0	" 3	"
"	"	85	"	"	"	"	"	0	" 2	"

SIXTH EXPERIMENT.

Bitch, 5 kilos. 3.7 grams *peptone* in 30 c.c. 0.7 per cent NaCl.
Injection lasted 40 seconds.
The normal blood coagulated in 3 minutes.

Blood withdrawn	5	minutes	after	injection	of	peptone	coagulated	in 6 hours.
"	"	9	"	"	"	"	"	"
"	"	15	"	"	"	"	"	"
"	"	24	"	"	"	"	"	1 hour.
"	"	55	"	"	"	"	"	45 minutes.
"	"	65	"	"	"	"	"	40 "
"	"	71	"	"	"	"	"	9 "

SEVENTH EXPERIMENT.

Bitch, 5.5 kilos. 2.75 grams *peptone* in 30 c.c. 0.7 per cent NaCl.
Injection lasted 30 seconds.
The normal blood coagulated in 1.5 minutes.

Blood withdrawn	3	minutes	after	injection	of	peptone	coagulated	in 3-10 hours.
"	"	7	"	"	"	"	"	"
"	"	13	"	"	"	"	"	"
"	"	18	"	"	"	"	"	"
"	"	39	"	"	"	"	"	"
"	"	50	"	"	"	"	"	76 minutes.
"	"	60	"	"	"	"	"	50 "
"	"	80	"	"	"	"	"	30 "
"	"	90	"	"	"	"	"	5 "
"	"	98	"	"	"	"	"	7 "

EIGHTH EXPERIMENT.

Dog, 5 kilos. Control experiment. 30 c.c. 0.7 per cent NaCl.
Injection lasted 30 seconds.
The normal blood coagulated in 5 minutes.

Blood withdrawn	2	minutes	after	injection	of	salt	solution	coagulated	in 3 min.
"	"	6	"	"	"	"	"	"	4 "

Fourteen minutes afterwards 3.0 grams *peptone* in 30 c.c. 0.7 per cent NaCl were injected.
Injection lasted 30 seconds.

Blood withdrawn	2	minutes	after	injection	of	peptone	coagulated	in 10-17 hours.
"	"	13	"	"	"	"	"	"
"	"	40	"	"	"	"	"	"
"	"	82	"	"	"	"	"	3 hrs. 16 min.
"	"	95	"	"	"	"	"	0 " 10 "

From these experiments it is very manifest that both deuteroalbumose and peptone, as formed from egg-albumin by papain, have a marked effect upon the coagulation of the blood. With the dosage employed, namely, fifty centigrams per kilo of body-weight, coagulation is retarded for periods ranging from thirty minutes to thirty-six hours.

Further, some of the experiments seemingly suggest that deuteroalbumose is somewhat more effective than pure peptone in retarding coagulation. It is likewise noticeable that this retarding effect upon coagulation is much more striking and also more permanent in some cases than in others, even though the conditions are apparently the same. Thus, in the second and third experiments, in which the dosage of deuteroalbumose per kilo is exactly the same, there is a marked difference in the character of the results, due, however, we believe, to differences in blood-pressure and to consequent differences in the rate of elimination through the kidneys. In connection with this last statement it is to be noted that in many of the experiments, with both albumose and peptone, the period of retardation shows a steady decrease (as in the fifth, seventh, and eighth experiments) until eventually, 50-100 minutes after the injection, the time of coagulation approaches somewhere near that of the normal blood.

What now is to be said regarding the relationship of these bodies in their action on blood-coagulation to the corresponding bodies of animal origin? Obviously, in considering this question little weight can be attached to results obtained with such products as Witte's so-called peptone, since the latter, as is well known, is a mixture of several albumoses with some peptone. Hence, the earlier results obtained with products in which the two classes of substances — albumoses and peptones — were not differentiated have in the present connection only a general interest.[1] Pollitzer,[2] on the other hand, working with the individual albumoses formed by pepsin-acid, found that while all of these substances prevented or delayed the coagulation of the blood, the primary albumoses were most effective, deuteroalbumose least so. Further, amphopeptone led to variable results, frequently wholly negative, while antipeptone as formed by trypsin was almost entirely wanting in any constant effects. Grosjean,[3] however, observed that the peptone formed in gastric digestion does retard coagulation, although its action is less vigorous than that of

[1] Schmidt-Mülheim: Beiträge zur Kenntniss des Peptons und seiner physiologischen Bedeutung. Du Bois-Reymond's Archiv f. Physiol., 1880, p. 33; Fano: Das Verhalten des Peptons und Tryptons gegen Blut und Lymphe. *Ibid.*, 1881, p. 277; Thompson: Contribution to the physiological effects of 'peptone' when injected into the circulation. Journal of physiology, 1896, xx. p. 455.

[2] Pollitzer: On the physiological action of peptones and albumoses. *Ibid.*, 1886, vii. p. 283.

[3] Grosjean: Recherches sur l'action physiologique de la propeptone et de la peptone. Archives de biologie, 1892, xii.

the albumoses. With antipeptone, Spiro and Ellinger[1] found that the effect produced was dependent entirely upon the dosage of peptone employed. Thus, with 0.6 gram per kilo of body-weight coagulation-time was reduced from eight to four minutes, while with 1.1 grams of peptone per kilo the blood was rendered non-coagulable. Lastly, Thompson[2] has reported that antipeptone in doses up to thirty centigrams per kilo tends to hasten the coagulation of the blood, while deuteroalbumose sometimes produces a retardation and sometimes a hastening of coagulation, apparently independent of the dosage.[3] It is obvious from these brief statements that any sharp comparison between the digestive products formed by papain and those resulting from the action of pepsin and trypsin is hardly possible. It is, however, seemingly true that the deuteroalbumose and peptone resulting from papain-digestion have a greater retarding effect upon blood-coagulation than the corresponding products formed by the animal enzymes. Thus, papain-peptone in doses of 0.5 gram per kilo never failed (in three experiments) to retard coagulation for 3-10 hours, while of antipeptone a dosage of 0.6 gram per kilo accelerates coagulation (Spiro and Ellinger). Further, papain-deuteroalbumose in doses of 0.33–0.5 gram per kilo invariably caused marked retardation of coagulation; far beyond anything reported by Thompson with deuteroalbumose in doses up to 0.3 gram per kilo. Any attempt at closer comparison in this direction would hardly be justified with our present knowledge. We would call special attention, however, to the tendency manifested in all of our experiments for the effect produced by papain-deuteroalbumose and peptone on the blood to pass gradually off, until finally, as in the fifth experiment, the coagulation-time may be considerably shorter than that of the normal blood. We attribute this result solely to the gradual elimination of the proteid, and as the rate of elimination varies with changes in blood-pressure, etc., produced by the substance, it follows that the duration of the effect upon the blood will vary not only with the do-

[1] Spiro and Ellinger: Der Antagonismus gerinnungsbefordernder und gerinnungshemmender Stoffe im Blute und die sogenannte Peptonimmunität. Zeitschr. f. physiol. Chem., 1897, xxiii, p. 135.

[2] Thompson: The physiological effects of peptone and its precursors when introduced into the circulation. Interim Report of a Committee consisting of Professors Schäfer, Sherrington, Boyce, and Thompson. Report by the Secretary, 1896–97.

[3] See also the papers on peptone and propeptone, by Gley and by Dastre in the Compt. rend. soc. de biologie, 1896.

sage given, but also with the period of its detention within the blood current. Lastly, the acceleration of coagulation observed 65–85 minutes after injection of the albumose (Experiment fifth) suggests that small doses of the substance may produce an effect quite the opposite of that produced by a large dose, as observed by Spiro and Ellinger with antipeptone. In conclusion, we see in these results nothing to warrant the assumption that the two papain products are widely different from ordinary digestive products of a like degree of hydration. They certainly do not differ from the corresponding products of pepsin or trypsin digestion more than the latter products differ among themselves. Thus, according to the experiments of Arthus and Huber,[1] of gelatose, 2 grams per kilo of body-weight are required to render the blood of the dog non-coagulable, while of caseose 1.5 grams per kilo are needed; amounts far larger than are required of an albumose formed from either egg-albumin or blood-fibrin. Indeed, we have observed in a single experiment with pure protogelatose that five grams of the substance (dissolved in water) introduced into the facial vein of a dog weighing between three and four kilos, hastened the rate of coagulation.

Elimination by the Kidneys. — As already stated, Neumeister has shown that when ordinary albumoses are introduced into the blood of the dog, they are eliminated in the urine more or less hydrated. The atmidalbumoses, on the contrary, he found were eliminated unchanged. What now is the behavior of the deuteroalbumose formed by papain when similarly injected? In the second experiment already detailed, in which a dog of 6.5 kilos was given 3.25 grams of papain-deuteroalbumose by injection into the facial vein, the bladder (empty at the beginning of the experiment) was found one hour after the injection distended with urine. The fluid, amounting to 150 c.c., was removed, filtered, and saturated, boiling hot, with ammonium sulphate. A heavy gummy precipitate resulted, which after being washed with a saturated solution of the ammonium salt, was dissolved in water and tested. It was composed of unaltered deuteroalbumose. On testing the filtrate from the latter precipitate, after again boiling with ammonium sulphate to ensure the complete removal of the deuteroalbumose, an intense biuret reaction was obtained, thus showing plainly the presence of a comparatively large amount of true peptone. In this experi-

[1] Arthus and Huber: Action des injections intraveineuses de produits de digestions peptique et tryptique de la gélatine et du caséum sur la coagulation du sang chez le chien. Arch. de physiol., 1897, 5, viii., p. 857.

ment, therefore, there was marked diuresis, accompanied by a rapid elimination of the deuteroalbumose, but most important of all, a large proportion of the eliminated albumose underwent hydration into true peptone during its transit from the blood to the urine. Such a result as this, however, is not always obtained. Thus, in the third experiment blood-pressure was greatly lowered, and an hour after the injection the bladder contained only a few drops of fluid, with which no distinct reaction for either albumose or peptone could be obtained. In harmony with these two results, the blood in the last experiment drawn 46 minutes after the injection did not coagulate within 18 hours, while in the first experiment, where elimination was comparatively rapid, the blood drawn 49 minutes after the injection coagulated in 38 minutes. Further, in the fifth experiment detailed above, 85 minutes after injection of the albumose, retardation of blood-coagulation was wholly at an end; indeed, coagulation took place more rapidly than prior to the injection. At this time the bladder was found distended with urine, and the latter gave a strong peptone reaction and a fair separation of albumose.[1] In the fourth experiment, there was no marked diuresis, but 76 minutes after the injection the bladder was half full of urine, the latter giving a strong reaction for peptone with only a trace of albumose. Thus, the results obtained in this connection certainly warrant the statement that whenever papain-deuteroalbumose undergoes elimination through the kidneys of the dog, it behaves in the same manner as an ordinary albumose, being transformed in great part into true peptone. It would seem, however, that injection of papain-deuteroalbumose is less liable to produce suspension of the renal secretion than injections of ordinary propeptone.[2]

With papain-peptone the elimination through the kidneys appeared less marked than with the albumose. Still, in all three experiments the bladder was found, 60–100 minutes after the injection, fairly well filled with urine, and on testing the latter a good biuret reaction for peptone was obtained. In no case was there any separation of an albumose precipitate on saturating the fluid with ammonium sulphate.

Influence on Blood-pressure.—Upon blood-pressure the albumose and peptone formed by papain from coagulated egg-albumin have

[1] In a recent preliminary communication (Proceed. Physiol. Soc., Nov. 13, 1897), Thompson has likewise reported that Witte's 'peptone' and Grosjean's peptone when injected into the jugular vein of dogs may lead to a marked increase in the quantity of urine accompanied by an excretion of part of the albumoses and peptone injected.

[2] Compare GROSJEAN: *Loc. cit.*

much the same general effect as that produced by ordinary proteoses and peptone. In all of our experiments, in which the pressure was recorded, the dosage of albumose or peptone per kilo was somewhat larger than that ordinarily employed, namely, 0.5 gram. With this dosage, however, there was in nearly every experiment a marked and rapid fall of pressure lasting for about ten minutes. Moreover, the extent of the fall was seemingly influenced somewhat by the rapidity of the injection, a fact which has been commented upon by Thompson.[1] Our experiments also incline us to the belief that the character of the result may be modified somewhat by the personality of the animal, independent of the dosage and the duration of the injection. The experiments in this direction, however, were not intended to be exhaustive, but simply to throw light upon the main problem as to whether the papain products differ radically from ordinary digestive products. In one or two instances the fall of pressure was hardly noticeable, and in these cases the elimination of the albumose through the urine was quite rapid. The two following experiments may be taken as typical of what was generally observed with deuteroalbumose.

In a dog weighing 7 kilos, narcotized by chloroform-ether, 3.5 grams of deuteroalbumose dissolved in 50 c.c. 0.7 per cent NaCl solution were injected into the right facial vein, the injection lasting 1 min. 15 sec. The blood-pressure was lowered immediately from 160 mm. Hg to about 25 mm. Within four minutes, however, the pressure began to rise gradually but steadily, and in ten minutes from the time of injection was approximately normal again.

A dog of 11.6 kilos, under chloroform-ether narcosis, was treated with 5.6 grams of the albumose by injection into the femoral vein, the injection lasting 45 seconds. Here, the pressure fell from about 150 mm. Hg to 100 mm. within one minute, gradually rising again to the normal in about seven minutes. In this experiment the fall of pressure was preceded by a slight rise amounting to 5 mm. This initial effect of the injection of deuteroalbumose upon blood-pressure, *i. e.* a slight rise, is in harmony with the observations of Thompson[2] with Witte's 'peptone.'

Similar experiments with pure papain-peptone gave corresponding results, namely, an immediate and rapid fall of pressure, the latter rising to the normal again in nine to twelve minutes.

[1] Thompson: Journal of physiology, 1896, xx, p. 460.

[2] Thompson: *loc. cit.*, p. 461.

THE GASTRIC INVERSION OF CANE-SUGAR BY HYDROCHLORIC ACID.

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IT has been the custom of Voit[1] for the past thirty-five years to demonstrate upon the lecture table that a 0.3 per cent hydrochloric acid solution acting upon cane-sugar at the temperature of the body causes a rapid inversion of the sugar, as may be shown by the acquired power to reduce Fehling's solution. That the stomach itself may cause this inversion has been noted by several authors, Röbner, Claude Bernard,[2] and others. Furthermore Seegen[3] has shown, on feeding cane-sugar to dogs and killing them two or three hours afterwards, that considerable quantities of cane-sugar with invert-sugar are to be found in the stomach, while only invert-sugar can be detected in the intestines. Seegen therefore concluded that the whole inversion of cane-sugar takes place in the stomach. In an experiment performed by one of us (L.)[4] under Prof. Voit's direction, 30 grams of cane sugar were given to a starving rabbit, and at the end of six and a half hours the animal was killed and the contents of the various intestinal segments analyzed. The figures in grams obtained are here reproduced:

	Cane-Sugar.	*Invert-Sugar.*
Stomach	0.269	2.356
Small Intestines	0.002	0.005
Cœcum	0.	2.167
Large Intestine	0.	0.102

It is seen here, with the exception of a minute quantity in the small intestine quite accountable as within the analytical error limit, that the cane-sugar is exclusively present in the stomach, and accompanying this cane-sugar is found nine times as much invert-sugar.

[1] Voit: Zeitschr. f. Biologie, 1891, xxviii, p. 268.

[2] For the Literature see Voit, *loc. cit.*

[3] Seegen: Archiv f. d. ges. Physiol., 1887, xl, p. 41.

[4] Voit: *loc. cit.*, p. 269.

The question to be solved by us was this: Is the acid of the gastric juice a sufficient agent to accomplish such inversion of cane-sugar as takes place in the stomach?

It is known that an enzyme of the intestines acts to convert cane-sugar into invert-sugar. This was shown by Miura[1] to be true of extracts of the mucosa of the small intestine, not only in the case of the rabbit and dog, but also of the mucosa of still-born infants, where presumably neither bacteria nor ferments introduced through the mouth could have been the cause of the inversion. In addition to the very complete literature cited by Miura may be mentioned the work of Mendel,[2] who found that the paralytic secretion from an intestinal fistula in the dog had the power of inverting a cane-sugar solution made antiseptic by one per cent of sodium fluoride. Although the presence of an inverting enzyme for cane-sugar in the small intestines is definitely proven, a similar enzyme within the mucosa of the stomach has not been found. Miura noticed only a very slight effect upon cane-sugar, when he used extracts from the stomachs of dogs, and none at all after using strips and extracts of the stomachs of still-born children. It may be added that Miura finds that the large intestine also has no influence upon the inversion of cane-sugar.

This being the known history of the behavior of saccharose within the digestive tract, our interest was excited to determine more in detail the extent of the action at the body temperature of hydrochloric acid on cane-sugar. The acids used for this purpose were in 0.1, 0.2 and 0.3 per cent solutions, comparable with the acidity of normal gastric juice. These digestions we have carried on for different periods of time.

The pure crystalline cane-sugar used by us showed, after inversion by boiling half an hour with a 0.1 per cent hydrochloric acid and subsequent neutralization, a reducing power equal to 100 per cent of the substance employed. A known quantity of this sugar in solution was brought into one beaker glass, and the necessary amount of dilute hydrochloric acid into another. Both beakers were placed in a thermostat at a temperature of between 37–40°, until they had acquired that temperature. The hydrochloric acid was then poured upon the sugar solution, and at the end of the required time the whole was exactly neutralized with sodium hydrate. As a matter of conven-

[1] MIURA: Zeitschr. f. Biologie, 1897, xxxii, p. 266.

[2] MENDEL: Archiv f. d. ges. Physiol., 1896, xliii, p. 434.

ience, we found it best to use as a supply a 0.5 per cent hydrochloric acid solution, and a sodium hydrate solution 1 c.c. of which would exactly neutralize 1 c.c. of the acid. The sugar solutions digested usually approximated one per cent, although five per cent solutions were also used, the total bulk being about 100 c.c. in each experiment. The sugar determinations were made in duplicate, according to the gravimetric method of Allihn. One hundred parts of invert-sugar found were calculated as ninety-five parts of cane-sugar.

The results obtained are given in tabular form below.

Time.	0.91 % Cane-Sugar.	0.95 % Sugar.	0.91 % Sugar.	5 % Sugar.	0.91 % Sugar.
	0.1 % HCl.	0.2 % HCl.	0.2 % HCl.	0.2 % HCl.	0.3 % HCl.
1 Hour . . .	....	14.0 %	....	15.5 %	22.2 %
2 " . . .	....	25.4	....	29.9	37.6
3 " . . .	....	30.9	....	34.2	49.5
4 " . . .	26.5 %	....	37.8 %	43.0	58.9
5 " . . .	....	....	47.5	59.6	62.4
7 " . . .	{ 40.6 / 39.3	....	76.8	69.2	79.3
10 " . . .	....	....	81.7	....	93.4
12 " . . .	63.8	....	86.4	....	94.1

The results show that the stronger the acid the greater the inversion. In general the same percentage inversion is obtained with a 5 per cent sugar solution as with a 0.91 per cent solution. The amount of the sugar inverted by the same acid is thus proportional to the strength of the sugar solution. While the acid is acting, the quantity of cane-sugar becomes continuously less in the solution. Hence a smaller amount is continuously being inverted. If we take the case of the 0.91 per cent sugar and the 0.1 per cent acid we find that 26.5 per cent of the sugar is inverted in four hours. If this proportionate decrease be maintained for twelve hours, 60.3 per cent of the sugar should be inverted, whereas, in fact, 63.8 per cent is found so changed. The process involved is dependent on Wilhelmy's law of chemical change.[1]

It seemed important to determine whether hydrochloric acid in

[1] Ostwald: Lehrbuch der allgemeinen Chemie, 1887, ii. p. 617.

chemical combination with proteid and the products of proteid digestion had this power of inverting cane-sugar. It was found that beaten and dialyzed white of egg digested with 0.3 per cent hydrochloric acid and pepsin until no reaction is given with tropæolin has no inverting power upon cane-sugar.[1] The sugar test was made after twice precipitating the proteid with acetic acid and absolute alcohol, in each case neutralizing and evaporating the alcohol. No reduction of Fehling's solution could be obtained.

If we now compare our results with the figures given for the experiment on the living rabbit, we find in the rabbit's stomach after six and a half hours, ten per cent of cane-sugar and ninety per cent of invert-sugar; while, on the other hand, in our beakers using 0.2 per cent and 0.3 per cent hydrochloric acid solutions, were found after seven hours between seventy and eighty per cent of invert-sugar, and twenty to thirty per cent of cane-sugar not inverted. Perhaps the higher inversion in the animal was due to the continual motion in the stomach, or possibly even to an acid of greater strength. At all events the results obtained in the beakers are not very divergent from those found in the animal.

We may perhaps draw the following picture of the fate of cane-sugar within the body. Sugar solutions (dextrose) up to 5 per cent according to Tappeiner[2] and Brandl[3] are not absorbed by the stomachs of dogs. In higher concentrations absorption takes place through the stomach wall, and the mucous membrane becomes flushed with blood and appears very red. If large quantities of cane-sugar be fed to rabbits[4] or to man[5] some of it may appear for a short time in the urine. This cane-sugar absorbed as such we now know from the investigations of F. Voit[6] is not burned in the system, but is quantitatively eliminated in the urine. Since only invert-sugar is to be found in the small intestine, it may safely be argued that the absorption of cane-sugar as such takes place only in the stomach, and there

[1] If unbeaten white of egg be used in the same way, inversion does take place. This we can explain only under the supposition that the digestive liquid penetrates the cells of the egg with difficulty, and that there is therefore free hydrochloric acid present, although on warming in the tropæolin test it has the opportunity of combining with the proteid.

[2] Tappeiner: Zeitschr. f. Biologie, 1880, xvi, p. 506.

[3] Brandl: Zeitschr. f. Biologie, 1892, xxix, p. 287.

[4] Voit: *loc. cit.*, p. 270.

[5] Moritz: Verhandl. d. X Congresses f. innere Medicin, 1891, pp. 492-501.

[6] Voit, F.: Münchener med. Wochenschrift, 1896, xliii, p. 887.

only when in large quantities. The strongly stimulated gastric mucosa must furnish a gastric juice containing much hydrochloric acid and hence capable of energetic inverting power. The enzyme present in the intestines has the office of quickly transforming into invert-sugar any of the cane-sugar which passes through the pylorus.

Proceeding one step farther, we find the statement of Minkowski [1] that levulose absorbed in pancreas diabetes may be converted into dextrose to the extent of fifty-three per cent. As much as seventy-five per cent of the cane-sugar fed may therefore be converted by the organism into dextrose, the ordinary sugar of the blood.

[1] MINKOWSKI: Archiv f. exper. Pathol. u. Pharmakol., 1893, xxxi. p. 157.